

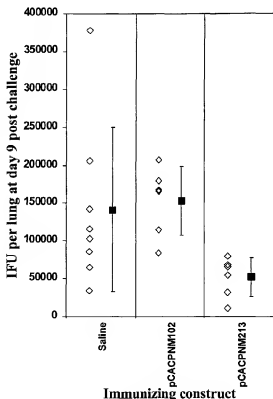
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[Continued on next page]

(54) Title: *CHLAMYDIA* ANTIGENS AND CORRESPONDING DNA FRAGMENTS AND USES THEREOF

(57) Abstract: The present invention provides nucleic acids, proteins and vectors for a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of *Chlamydia*, specifically *C. pneumoniae*. The method employs a vector containing a nucleotide sequence encoding a polypeptide of a strain of *Chlamydia pneumoniae* operably linked to a promoter to effect expression of the gene product in the host. The polypeptides are derived from *C. pneumoniae* and are selected from an ATP-binding cassette protein, a secretory locus ORF, an endopeptidase, a protease, a metalloprotease, CLP protease ATPase, a CLP protease subunit, a transacylase / transpeptidase, a CLP protease and thioredoxin. Modifications are possible within the scope of this invention.

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**TITLE OF INVENTION**

CHLAMYDIA ANTIGENS AND CORRESPONDING DNA FRAGMENTS  
AND USES THEREOF

**REFERENCE TO RELATED APPLICATIONS**

- 5           This application claims the benefit of U.S.  
Provisional Application Nos. 60/202,672, filed May 8, 2000;  
60/207,852 filed May 30, 2000; 60/211,801, 60/212,044,  
60/211,797, 60/211,796 and 60/211,798 filed June 16, 2000; and  
60/235,335, 60/235,361 and 60/235,398 filed September 26, 2000.

10 **FIELD OF INVENTION**

- The present invention relates to a number of  
*Chlamydia* antigens, including an ATP-binding cassette protein,  
a secretory locus ORF, an endopeptidase, a protease, a  
metalloprotease, CLP protease ATPase, a CLP protease subunit, a  
15 translycolase / transpeptidase, a CLPc protease and  
thioredoxin, and their corresponding DNA molecules, for the  
prevention and treatment of *Chlamydia* infection in mammals.

**BACKGROUND OF THE INVENTION**

- Chlamydiae* are prokaryotes. They exhibit morphologic  
20 and structural similarities to gram-negative bacteria including  
a trilaminar outer membrane, which contains lipopolysaccharide  
and several membrane proteins that are structurally and  
functionally analogous to proteins found in *E. coli*. They are  
obligate intra-cellular parasites with a unique biphasic life  
25 cycle consisting of a metabolically inactive but infectious  
extracellular stage and a replicating but non-infectious  
intracellular stage. The replicative stage of the life-cycle  
takes place within a membrane-bound inclusion which sequesters  
the bacteria away from the cytoplasm of the infected host cell.

*C. pneumoniae* is a common human pathogen, originally described as the TWAR strain of *Chlamydia psittaci* but subsequently recognised to be a new species. *C. pneumoniae* is antigenically, genetically and morphologically distinct from  
5 other *Chlamydia* species (*C. trachomatis*, *C. pecorum* and *C. psittaci*). It shows 10% or less DNA sequence homology with either of *C. trachomatis* or *C. psittaci*.

In general, all chlamydiae share a common developmental microbiology and appear to share a common  
10 immunobiology. Genome analysis shown that over 80% of *C. pneumoniae* and *C. trachomatis* protein-coding genes are orthologs that share a similar genome organization.

*C. pneumoniae* is the third most common cause of community acquired pneumonia, only less frequent than  
15 *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477). It can also cause upper respiratory tract symptoms and disease, including bronchitis and sinusitis (Grayston et  
20 al. (1995) Journal of Infectious Diseases 168:1231; Grayston et al (1990) Journal of Infectious Diseases 161:618-625; Marrie (1993) Clinical Infectious Diseases. 18:501-513; Wang et al (1986) Chlamydial infections Cambridge University Press, Cambridge. p. 329. The great majority of the adult population  
25 (over 60%) has antibodies to *C. pneumoniae* (Wang et al (1986) Chlamydial infections. Cambridge University Press, Cambridge. p. 329), indicating past infection which was unrecognized or asymptomatic.

*C. pneumoniae* infection usually presents as an acute  
30 respiratory disease (i.e., cough, sore throat, hoarseness, and fever; abnormal chest sounds on auscultation). For most patients, the cough persists for 2 to 6 weeks, and recovery is

slow. In approximately 10% of these cases, upper respiratory tract infection is followed by bronchitis or pneumonia. Furthermore, during a *C. pneumoniae* epidemic, subsequent co-infection with pneumococcus has been noted in about half of these pneumonia patients, particularly in the infirm and the elderly. As noted above, there is increasing evidence that *C. pneumoniae* infection is also linked to diseases other than respiratory infections.

The reservoir for the organism is presumably people. In contrast to *C. psittaci* infections, there is no known bird or animal reservoir. Transmission has not been clearly defined. It may result from direct contact with secretions, from fomites, or from airborne spread. There is a long incubation period, which may last for many months. Based on analysis of epidemics, *C. pneumoniae* appears to spread slowly through a population (case-to-case interval averaging 30 days) because infected persons are inefficient transmitters of the organism. Susceptibility to *C. pneumoniae* is universal. Reinfections occur during adulthood, following the primary infection as a child. *C. pneumoniae* appears to be an endemic disease throughout the world, noteworthy for superimposed intervals of increased incidence (epidemics) that persist for 2 to 3 years. *C. trachomatis* infection does not confer cross-immunity to *C. pneumoniae*. Infections are easily treated with oral antibiotics, tetracycline or erythromycin (2 g/d, for at least 10 to 14 d). A recently developed drug, azithromycin, is highly effective as a single-dose therapy against chlamydial infections.

In most instances, *C. pneumoniae* infection is often mild and without complications, and up to 90% of infections are subacute or unrecognized. Among children in industrialized countries, infections have been thought to be rare up to the age of 5 y, although a recent study (E Normann et al, *Chlamydia*

*pneumoniae* in children with acute respiratory tract infections, Acta Paediatrica, 1998, Vol 87, Iss 1, pp 23-27) has reported that many children in this age group show PCR evidence of infection despite being seronegative, and estimates a  
5 prevalence of 17-19% in 2-4 y olds. In developing countries, the seroprevalence of *C. pneumoniae* antibodies among young children is elevated, and there are suspicions that *C. pneumoniae* may be an important cause of acute lower respiratory tract disease and mortality for infants and children in  
10 tropical regions of the world.

From seroprevalence studies and studies of local epidemics, the initial *C. pneumoniae* infection usually happens between the ages of 5 and 20 y. In the USA, for example, there are estimated to be 30,000 cases of childhood pneumonia each  
15 year caused by *C. pneumoniae*. Infections may cluster among groups of children or young adults (e.g., school pupils or military conscripts).

*C. pneumoniae* causes 10 to 25% of community-acquired lower respiratory tract infections (as reported from Sweden,  
20 Italy, Finland, and the USA). During an epidemic, *C. pneumoniae* infection may account for 50 to 60% of the cases of pneumonia. During these periods, also, more episodes of mixed infections with *S. pneumoniae* have been reported.

Reinfection during adulthood is common; the clinical  
25 presentation tends to be milder. Based on population seroprevalence studies, there tends to be increased exposure with age, which is particularly evident among men. Some investigators have speculated that a persistent, asymptomatic *C. pneumoniae* infection state is common.

30 In adults of middle age or older, *C. pneumoniae* infection may progress to chronic bronchitis and sinusitis. A study in the USA revealed that the incidence of pneumonia

caused by *C. pneumoniae* in persons younger than 60 years is 1 case per 1,000 persons per year; but in the elderly, the disease incidence rose three-fold. *C. pneumoniae* infection rarely leads to hospitalization, except in patients with an underlying illness.

Of considerable importance is the association of atherosclerosis and *C. pneumoniae* infection. There are several epidemiological studies showing a correlation of previous infections with *C. pneumoniae* and heart attacks, coronary artery and carotid artery disease (Saikku et al. (1988) Lancet;ii:983-986; Thom et al. (1992) JAMA 268:68-72; Linnanmaki et al. (1993), Circulation 87:1030; Saikku et al. (1992) Annals Internal Medicine 116:273-287; Melnick et al (1993) American Journal of Medicine 95:499). Moreover, the organisms has been detected in atheromas and fatty streaks of the coronary, carotid, peripheral arteries and aorta (Shor et al. (1992) South African. Medical Journal 82:158-161; Kuo et al. (1993) Journal of Infectious Diseases 167:841-849; Kuo et al. (1993) Arteriosclerosis and Thrombosis 13:1501-1504; Campbell et al (1995) Journal of Infectious Diseases 172:585; Chiu et al. Circulation, 1997. Circulation. 96:2144-2148). Viable *C. pneumoniae* has been recovered from the coronary and carotid artery (Ramirez et al (1996) Annals of Internal Medicine 125:979-982; Jackson et al. 1997. J. Infect. Dis. 176:292-295). Furthermore, it has been shown that *C. pneumoniae* can induce changes of atherosclerosis in a rabbit model (Fong et al. 1997. Journal of Clinical Microbiology 35:48 and Laitinen et al. 1997. Infect. Immun. 65:4832-4835). Taken together, these results indicate that it is highly probable that *C. pneumoniae* can cause atherosclerosis in humans, though the epidemiological importance of chlamydial atherosclerosis remains to be demonstrated.

A number of recent studies have also indicated an association between *C. pneumoniae* infection and asthma. Infection has been linked to wheezing, asthmatic bronchitis, adult-onset asthma and acute exacerbations of asthma in adults, and small-scale studies have shown that prolonged antibiotic treatment was effective at greatly reducing the severity of the disease in some individuals (Hahn DL, et al. Evidence for *Chlamydia pneumoniae* infection in steroid-dependent asthma. Ann Allergy Asthma Immunol. 1998 Jan; 80(1): 45-49.; Hahn DL, et al. Association of *Chlamydia pneumoniae* IgA antibodies with recently symptomatic asthma. Epidemiol Infect. 1996 Dec; 117(3): 513-517; Bjornsson E, et al. Serology of *chlamydia* in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis. 1996; 28(1): 63-69.; Hahn DL. Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before-after trial. J Fam Pract. 1995 Oct; 41(4): 345-351.; Allegra L, et al. Acute exacerbations of asthma in adults: role of *Chlamydia pneumoniae* infection. Eur Respir J. 1994 Dec; 7(12): 2165-2168.; Hahn DL, et al. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA. 1991 Jul 10; 266(2): 225-230).

In light of these results a protective vaccine against *C. pneumoniae* infection would be of considerable importance. There is not yet an effective vaccine for any human chlamydial infection. It is conceivable that an effective vaccine can be developed using physically or chemically inactivated *Chlamydiae*. However, such a vaccine does not have a high margin of safety. In general, safer vaccines are made by genetically manipulating the organism by attenuation or by recombinant means.

A disease associated with *C. trachomatis* infection is trachoma, a sequela of ocular infection. This disease continues to be a major cause of preventable blindness, with an



estimated 500 million cases of active trachoma worldwide (seven million include blindness from conjunctival scarring and eyelid deformities). In the last two decades, genital chlamydial infection has been identified as a major public health problem because of the recognition that chlamydial infection is associated with disease syndromes such as non-gonococcal urethritis, mucopurulent cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy, and tubal infertility. The World Health Organization estimated 89 million new cases of genital chlamydial infections worldwide in 1995. In the United States, each year an estimated four million new cases occur and 50,000 women become infertile as a result of infection.

Studies with *C. trachomatis* and *C. psittaci* indicate that safe and effective vaccine against *Chlamydia* is an attainable goal. For example, mice which have recovered from a lung infection with *C. trachomatis* are protected from infertility induced by a subsequent vaginal challenge (Pal et al. (1996) Infection and Immunity. 64:5341). Similarly, sheep immunized with inactivated *C. psittaci* were protected from subsequent chlamydial-induced abortions and stillbirths (Jones et al. (1995) Vaccine 13:715). In a mouse model, protection from chlamydial infections has been associated with Th1 immune responses, particularly CD8+ CTL response (Rottenberg et al. 1999. J. Immunol. 162:2829-2836 and Penttila et al. 1999. Immunology. 97:490-496) and it is unlikely that similar responses will need to be induced in humans to confer protection. However, antigens able to elicit a protective immune response against *C. pneumoniae* are largely unknown. The presence of sufficiently high titres of neutralising antibody at mucosal surfaces can also exert a protective effect (Cotter et al. (1995) Infection and Immunity 63:4704).

Antigenic variation within the species *C. pneumoniae* is not well documented due to insufficient genetic information,

though variation is expected to exist based on *C. trachomatis*. Serovars of *C. trachomatis* are defined on the basis of antigenic variation in the major outer membrane protein (MOMP), but published *C. pneumoniae* MOMP gene sequences show no

5 variation between several diverse isolates of the organism (Campbell et al. Infection and Immunity (1990) 58:93; McCafferty et al. Infection and Immunity (1995) 63:2387-9; Gaydos et al. Infection and Immunity. (1992) 60(12):5319-5323). The gene encoding a 76 kDa antigen has been cloned from a

10 single strain of *C. pneumoniae* and the sequence published (Perez Melgosa et al. Infection and Immunity. (1994) 62:880). An operon encoding the 9 kDa and 60 kDa cyteine-rich outer membrane protein genes has been described (Watson et al., Nucleic Acids Res (1990) 18:5299; Watson et al., Microbiology

15 (1995) 141:2489). Many antigens recognized by immune sera to *C. pneumoniae* are conserved across all *chlamydiae*, but 98 kDa, 76 kDa and several other proteins may be *C. pneumoniae*-specific (Knudsen et al. Infect. Immun. 1999. 67:375-383; Perez Melgosa et al. Infection and Immunity. 1994. 62:880; Melgosa et al.,

20 FEMS Microbiol Lett 1993. 112 :199; Campbell et al., J. Clin. Microbiol. 1990. 28 :1261; Iijima et al., J. Clin. Microbiol. 1994. 32:583). Antisera to 76kDa and 54kDa antigens have been reported to neutralize *C. pneumoniae* in vitro (Perez Melgosa et al. 1994. Infect. Immun. 62:880-886 and Wiedman-Al-Ahmad et al.

25 1997. Clin. Diagn. Lab. Immunol. 4:700-704). An assessment of the number and relative frequency of any *C. pneumoniae* serotypes, and the defining antigens, is not yet possible. The entire genome sequence of *C. pneumoniae* strain CWL-029 is now known (<http://chlamydia-www.berkeley.edu:4231/>) and as further

30 sequences become available a better understanding of antigenic variation may be gained.

Many antigens recognised by immune sera to *C. pneumoniae* are conserved across all *chlamydiae*, but 98kDa,

76 kDa and 54 kDa proteins appear to be *C. pneumoniae*-specific (Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477; Marrie (1993) Clinical Infectious Diseases. 18:501-513; Wiedmann-Al-Ahmad M, et al. Reactions of polyclonal  
5 and neutralizing anti-p54 monoclonal antibodies with an isolated, species-specific 54-kilodalton protein of *Chlamydia pneumoniae*. Clin Diagn Lab Immunol. 1997 Nov; 4(6): 700-704).

Immunoblotting of isolates with sera from patients does show variation of blotting patterns between isolates,  
10 indicating that serotypes *C. pneumoniae* may exist (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Ramirez et al (1996) Annals of Internal Medicine 125:979-982). However, the results are potentially confounded by the infection status of the patients, since immunoblot profiles of a patient's sera  
15 change with time post-infection. An assessment of the number and relative frequency of any serotypes, and the defining antigens, is not yet possible.

The use of DNA immunization to elicit a protective immune response in Balb/c mice against pulmonary infection with  
20 the mouse pneumonitis (MoPn) strain of *Chlamydia trachomatis* has recently been described (Zhang et al. 1997. J. Infect. Dis. 166:1035-1040 and Zhang et al. 1999. Immunology. 96:314-321). Recently the genome sequence from *C. pneumoniae* strain CM1 (ATCC #1360-VR) has been disclosed by Griffais in WO99/27105  
25 on June 3, 1999.

Accordingly, a need exists for identifying and isolating polynucleotide sequences of *C. pneumoniae* for use in preventing and treating *Chlamydia* infection.

**SUMMARY OF THE INVENTION**

The present invention provides purified and isolated polynucleotide molecules that encode a *Chlamydia* polypeptide selected from: an ATP-binding cassette protein, a secretory locus ORF, an endopeptidase, a protease, a metalloprotease, CLP  
5 protease ATPase, a CLP protease subunit, a translycolase / transpeptidase, a CLPc protease and thioredoxin. The polynucleotide molecules can be used in methods to prevent, treat, and diagnose *Chlamydia* infection. In one embodiment of  
10 the invention, the polynucleotide molecules is DNA that encode a polypeptide of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

Another form of the invention provides polypeptides corresponding to an isolated DNA molecule. Amino acid  
15 sequences of the corresponding encoded polypeptides are shown in one embodiment as SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

Those skilled in the art will readily understand that the invention, having provided the polynucleotide sequences  
20 encoding *Chlamydia* polypeptides, also provides polynucleotides encoding fragments derived from such polypeptides. Moreover, the invention is understood to provide mutants and derivatives of such polypeptides and fragments derived therefrom, which result from the addition, deletion, or substitution of non-  
25 essential amino acids as described herein. Those skilled in the art would also readily understand that the invention, having provided the polynucleotide sequences encoding *Chlamydia* polypeptides, further provides monospecific antibodies that specifically bind to such polypeptides.

30 The present invention has wide application and includes expression cassettes, vectors, and cells transformed or transfected with the polynucleotides of the invention.

Accordingly, the present invention further provides (i) a method for producing a polypeptide of the invention in a recombinant host system and related expression cassettes, vectors, and transformed or transfected cells; (ii) a vaccine, or a live vaccine vector such as a pox virus, *Salmonella typhimurium*, or *Vibrio cholerae* vector, containing a polypeptide or a polynucleotide of the invention, such vaccines and vaccine vectors being useful for, e.g., preventing and treating *Chlamydia* infection, in combination with a diluent or carrier, and related pharmaceutical compositions and associated therapeutic and/or prophylactic methods; (iii) a therapeutic and/or prophylactic use of an RNA or DNA molecule of the invention, either in a naked form or formulated with a delivery vehicle, a polypeptide or combination of polypeptides, or a monospecific antibody of the invention, and related pharmaceutical compositions; (iv) a method for diagnosing the presence of *Chlamydia* in a biological sample, which can involve the use of a DNA or RNA molecule, a monospecific antibody, or a polypeptide of the invention; and (v) a method for purifying a polypeptide of the invention by antibody-based affinity chromatography.

One aspect of the invention provides a vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence set forth in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid

sequence to the polypeptide encoded by any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

Another aspect of the invention provides a vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1 shows the nucleotide sequence of the gene encoding an ATP-binding cassette (SEQ ID No: 1) and the deduced amino acid sequence of the ATP-binding cassette from *Chlamydia pneumoniae* (SEQ ID No: 2).

Figure 2 shows the nucleotide sequence of the gene encoding a secretory locus ORF (SEQ ID No: 3) and the deduced amino acid sequence of the secretory locus ORF from *Chlamydia pneumoniae* (SEQ ID No: 4).

Figure 3 shows the nucleotide sequence of the gene encoding an endopeptidase (SEQ ID No: 5) and the deduced amino acid sequence of the endopeptidase from *Chlamydia pneumoniae* (SEQ ID No: 6).

Figure 4 shows the nucleotide sequence of the gene encoding a protease (SEQ ID No: 7) and the deduced amino acid sequence of the protease from *Chlamydia pneumoniae* (SEQ ID No: 8).

Figure 5 shows the nucleotide sequence of the gene encoding a metalloprotease (SEQ ID No: 9) and the deduced amino acid sequence of the metalloprotease from *Chlamydia pneumoniae* (SEQ ID No: 10).

Figure 6 shows the nucleotide sequence of the gene encoding CLP protease ATPase (SEQ ID No: 11) and the deduced

amino acid sequence of the CLP protease ATPase from *Chlamydia pneumoniae* (SEQ ID No: 12).

Figure 7 shows the nucleotide sequence of the gene encoding a CLP protease subunit (SEQ ID No: 13) and the deduced  
5 amino acid sequence of the CLP protease subunit from *Chlamydia pneumoniae* (SEQ ID No: 14).

Figure 8 shows the nucleotide sequence of the gene encoding a transglycolase / transpeptidase (SEQ ID No: 15) and the deduced amino acid sequence of the transglycolase /  
10 transpeptidase from *Chlamydia pneumoniae* (SEQ ID No: 16).

Figure 9 shows the nucleotide sequence of the gene encoding a CLPc protease (SEQ ID No: 17) and the deduced amino acid sequence of the CLPc protease from *Chlamydia pneumoniae* (SEQ ID No: 18).

15 Figure 10 shows the nucleotide sequence of the gene encoding thioredoxin (SEQ ID No: 19) and the deduced amino acid sequence of thioredoxin from *Chlamydia pneumoniae* (SEQ ID No: 20).

Figure 11 shows the restriction enzyme analysis of  
20 the *C. pneumoniae* gene encoding an ATP-binding cassette.

Figure 12 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a secretory locus ORF.

Figure 13 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding an endopeptidase.

25 Figure 14 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a protease.

Figure 15 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a metalloprotease.



Figure 16 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding CLP protease ATPase.

Figure 17 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a CLP protease subunit.

5           Figure 18 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a translycolase / transpeptidase.

Figure 19 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding a CLPc protease.

10           Figure 20 shows the restriction enzyme analysis of the *C. pneumoniae* gene encoding thioredoxin.

Figure 21 shows the construction and elements of plasmid pCACPNM213.

15           Figure 22 shows the construction and elements of plasmid pCACPNM882.

Figure 23 shows the construction and elements of plasmid pCACPNM208.

Figure 24 shows the construction and elements of plasmid pCACPNM1096.

20           Figure 25 shows the construction and elements of plasmid pCACPNM1097.

Figure 26 shows the construction and elements of plasmid pCACPNM908.

25           Figure 27 shows the construction and elements of plasmid pCACPNM909.

Figure 28 shows the construction and elements of plasmid pCACPNM440.

Figure 29 shows the construction and elements of plasmid pCACPNM459.

Figure 30 shows the construction and elements of plasmid pCACPNM708.

5           Figure 31 illustrates protection against *C. pneumoniae* infection by pCACPNM213 following DNA immunization.

Figure 32 illustrates protection against *C. pneumoniae* infection by pCACPNM882 following DNA immunization.

10           Figure 33 illustrates protection against *C. pneumoniae* infection by pCACPNM208 following DNA immunization.

Figure 34 illustrates protection against *C. pneumoniae* infection by pCACPNM1096 following DNA immunization.

Figure 35 illustrates protection against *C. pneumoniae* infection by pCACPNM1097 following DNA immunization.

15           Figure 36 illustrates protection against *C. pneumoniae* infection by pCACPNM908 following DNA immunization.

Figure 37 illustrates protection against *C. pneumoniae* infection by pCACPNM909 following DNA immunization.

20           Figure 38 illustrates protection against *C. pneumoniae* infection by pCACPNM2440 following DNA immunization.

Figure 39 illustrates protection against *C. pneumoniae* infection by pCACPNM459 following DNA immunization.

Figure 40 illustrates protection against *C. pneumoniae* infection by pCACPNM708 following DNA immunization.

DETAILED DESCRIPTION OF INVENTION

Open reading frames (ORFs) encoding a number of Chlamydial proteins have been identified from the *C. pneumoniae* genome. These proteins include an ATP-binding cassette protein, a secretory locus ORF, an endopeptidase, a protease, a metalloprotease, CLP protease ATPase, a CLP protease subunit, a transglycolase / transpeptidase, a CLPc protease and thioredoxin. The gene encoding each of these polypeptides has been inserted into an expression plasmid and shown to confer immune protection against chlamydial infection. Accordingly, any one of these and related polypeptides can be used to prevent and treat *Chlamydia* infection.

According to a first aspect of the invention, isolated polynucleotides are provided which encode *Chlamydia* polypeptides, whose amino acid sequences are shown in SEQ ID No: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

The term "isolated polynucleotide" is defined as a polynucleotide removed from the environment in which it naturally occurs. For example, a naturally-occurring DNA molecule present in the genome of a living bacteria or as part of a gene bank is not isolated, but the same molecule separated from the remaining part of the bacterial genome, as a result of, e.g., a cloning event (amplification), is isolated. Typically, an isolated DNA molecule is free from DNA regions (e.g., coding regions) with which it is immediately contiguous at the 5' or 3' end, in the naturally occurring genome. Such isolated polynucleotides may be part of a vector or a composition and still be defined as isolated in that such a vector or composition is not part of the natural environment of such polynucleotide.

The polynucleotide of the invention is either RNA or DNA (cDNA, genomic DNA, or synthetic DNA), or modifications,

variants, homologs or fragments thereof. The DNA is either double-stranded or single-stranded, and, if single-stranded, is either the coding strand or the non-coding (anti-sense) strand. Any one of the sequences that encode the polypeptides of the invention as shown in any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 is (a) a coding sequence, (b) a ribonucleotide sequence derived from transcription of (a), or (c) a coding sequence which uses the redundancy or degeneracy of the genetic code to encode the same polypeptides. By "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Both terms are used interchangeably in the present application.

Consistent with the first aspect of the invention, amino acid sequences are provided which are homologous to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. As used herein, "homologous amino acid sequence" is any polypeptide which is encoded, in whole or in part, by a nucleic acid sequence which hybridizes at 25-35°C below critical melting temperature ( $T_m$ ), to any portion of the nucleic acid sequence of any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19. A homologous amino acid sequence is one that differs from an amino acid sequence shown in any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 by one or more conservative amino acid substitutions. Such a sequence also encompasses serotypic variants (defined below) as well as sequences containing deletions or insertions which retain inherent characteristics of the polypeptide such as immunogenicity. Preferably, such a sequence is at least 75%, preferably at least 78%, more preferably at least 80%, even more preferably at least 85%, 88% or 90%, and most preferably at least 93%, 95% or 98% identical to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

Homologous amino acid sequences include sequences that are identical or substantially identical to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. By "amino acid sequence substantially identical" is meant a sequence that is  
5 at least 90%, preferably 95%, more preferably 97%, and most preferably 99% identical to an amino acid sequence of reference and that preferably differs from the sequence of reference by a majority of conservative amino acid substitutions.

Conservative amino acid substitutions are  
10 substitutions among amino acids of the same class. These classes include, for example, amino acids having uncharged polar side chains, such as asparagine, glutamine, serine, threonine, and tyrosine; amino acids having basic side chains, such as lysine, arginine, and histidine; amino acids having  
15 acidic side chains, such as aspartic acid and glutamic acid; and amino acids having nonpolar side chains, such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and cysteine.

Homology is measured using sequence analysis software  
20 such as Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705. Amino acid sequences are aligned to maximize identity. Gaps may be artificially introduced into the sequence to attain proper  
25 alignment. Once the optimal alignment has been set up, the degree of homology is established by recording all of the positions in which the amino acids of both sequences are identical, relative to the total number of positions.

Homologous polynucleotide sequences are defined in a  
30 similar way. Preferably, a homologous sequence is one that is at least 45%, more preferably 50%, 55%, 60%, 65%, 70%, 75%, 80%, and even more preferably 85%, 87%, 90%, 93%, 96% and most

preferably 99% identical to the coding sequence of any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19.

Consistent with the first aspect of the invention, polypeptides having a sequence homologous to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 include naturally-occurring allelic variants, as well as mutants or any other non-naturally occurring variants that retain the inherent characteristics of the polypeptide of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

As is known in the art, an allelic variant is an alternate form of a polypeptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does not alter the biological function of the polypeptide. By "biological function" is meant the function of the polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. Biological function is distinct from antigenic property. A polypeptide can have more than one biological function.

Allelic variants are very common in nature. For example, a bacterial species such as *C. pneumoniae*, is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence (and polynucleotide sequence) that is not identical in each of the strains. Despite this variation, an immune response directed generally against many allelic variants has been demonstrated. In studies of the *Chlamydial* MOMP antigen, cross-strain antibody binding plus neutralization of infectivity occurs despite amino acid

sequence variation of MOMP from strain to strain, indicating that the MOMP, when used as an immunogen, is tolerant of amino acid variations.

Polynucleotides encoding homologous polypeptides or  
5 allelic variants are retrieved by polymerase chain reaction (PCR) amplification of genomic bacterial DNA extracted by conventional methods. This involves the use of synthetic oligonucleotide primers matching upstream and downstream of the 5' and 3' ends of the encoding domain. Suitable primers are  
10 designed according to the nucleotide sequence information provided in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19. The procedure is as follows: a primer is selected which consists of 10 to 40, preferably 15 to 25 nucleotides. It is advantageous to select primers containing C and G  
15 nucleotides in a proportion sufficient to ensure efficient hybridization; i.e., an amount of C and G nucleotides of at least 40%, preferably 50% of the total nucleotide content. A standard PCR reaction contains typically 0.5 to 5 Units of Taq DNA polymerase per 100  $\mu$ L, 20 to 200  $\mu$ M deoxynucleotide each,  
20 preferably at equivalent concentrations, 0.5 to 2.5 mM magnesium over the total deoxynucleotide concentration,  $10^5$  to  $10^6$  target molecules, and about 20 pmol of each primer. About 25 to 50 PCR cycles are performed, with an annealing temperature 15°C to 5°C below the true  $T_m$  of the primers. A  
25 more stringent annealing temperature improves discrimination against incorrectly annealed primers and reduces incorporation of incorrect nucleotides at the 3' end of primers. A denaturation temperature of 95°C to 97°C is typical, although higher temperatures may be appropriate for dematuration of G+C-  
30 rich targets. The number of cycles performed depends on the starting concentration of target molecules, though typically more than 40 cycles is not recommended as non-specific background products tend to accumulate.

An alternative method for retrieving polynucleotides encoding homologous polypeptides or allelic variants is by hybridization screening of a DNA or RNA library. Hybridization procedures are well-known in the art and are described in

5 Ausubel *et al.*, (Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994), Silhavy *et al.* (Silhavy *et al.* Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, 1984), and Davis *et al.* (Davis *et al.* A Manual for Genetic Engineering: Advanced Bacterial Genetics,

10 Cold Spring Harbor Laboratory Press, 1980)). Important parameters for optimizing hybridization conditions are reflected in a formula used to obtain the critical melting temperature above which two complementary DNA strands separate from each other (Casey & Davidson, Nucl. Acid Res. (1977)

15 4:1539). For polynucleotides of about 600 nucleotides or larger, this formula is as follows:  $T_m = 81.5 + 0.41 \times (\% \text{ G+C}) + 16.6 \log (\text{cation ion concentration}) - 0.63 \times (\% \text{ formamide}) - 600/\text{base number}$ . Under appropriate stringency conditions, hybridization temperature ( $T_h$ ) is approximately 20 to 40°C, 20

20 to 25°C, or, preferably 30 to 40°C below the calculated  $T_m$ . Those skilled in the art will understand that optimal temperature and salt conditions can be readily determined.

For the polynucleotides of the invention, stringent conditions are achieved for both pre-hybridizing and

25 hybridizing incubations (i) within 4-16 hours at 42°C, in 6 x SSC containing 50% formamide, or (ii) within 4-16 hours at 65°C in an aqueous 6 x SSC solution (1 M NaCl, 0.1 M sodium citrate (pH 7.0)). Typically, hybridization experiments are performed at a temperature from 60 to 68°C, e.g. 65°C. At such a

30 temperature, stringent hybridization conditions can be achieved in 6xSSC, preferably in 2xSSC or 1xSSC, more preferably in 0.5xSSC, 0.3xSSC or 0.1xSSC (in the absence of formamide). 1xSSC contains 0.15 M NaCl and 0.015 M sodium citrate.



Useful homologs and fragments thereof that do not occur naturally are designed using known methods for identifying regions of an antigen that are likely to tolerate amino acid sequence changes and/or deletions. As an example, 5 homologous polypeptides from different species are compared; conserved sequences are identified. The more divergent sequences are the most likely to tolerate sequence changes. Homology among sequences may be analyzed using, as an example, the BLAST homology searching algorithm of Altschul et al., 10 Nucleic Acids Res.; 25:3389-3402 (1997). Alternatively, sequences are modified such that they become more reactive to T- and/or B-cells, based on computer-assisted analysis of probable T- or B-cell epitopes. Yet another alternative is to mutate a particular amino acid residue or sequence within the 15 polypeptide *in vitro*, then screen the mutant polypeptides for their ability to prevent or treat *Chlamydia* infection according to the method outlined below.

A person skilled in the art will readily understand that by following the screening process of this invention, it 20 will be determined without undue experimentation whether a particular homolog of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 may be useful in the prevention or treatment of *Chlamydia* infection. The screening procedure comprises the steps:

25 (i) immunizing an animal, preferably mouse, with the test homolog or fragment;

(ii) inoculating the immunized animal with *Chlamydia*;  
and

(iii) selecting those homologs or fragments which 30 confer protection against *Chlamydia*.

By "conferring protection" is meant that there is a reduction in severity of any of the effects of *Chlamydia* infection, in comparison with a control animal which was not immunized with the test homolog or fragment.

5 Consistent with the first aspect of the invention, polypeptide derivatives are provided that are partial sequences of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20, partial sequences of polypeptide sequences homologous to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20,  
10 polypeptides derived from full-length polypeptides by internal deletion, and fusion proteins.

It is an accepted practice in the field of immunology to use fragments and variants of protein immunogens as vaccines, as all that is required to induce an immune response  
15 to a protein is a small (e.g., 8 to 10 amino acid) immunogenic region of the protein. Various short synthetic peptides corresponding to surface-exposed antigens of pathogens other than *Chlamydia* have been shown to be effective vaccine antigens against their respective pathogens, e.g. an 11 residue peptide  
20 of murine mammary tumor virus (Casey & Davidson, Nucl. Acid Res. (1977) 4:1539), a 16-residue peptide of Semliki Forest virus (Snijders et al., 1991. J. Gen. Virol. 72:557-565), and two overlapping peptides of 15 residues each from canine parvovirus (Langeveld et al., Vaccine 12(15):1473-1480, 1994)

25 Accordingly, it will be readily apparent to one skilled in the art, having read the present description, that partial sequences of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 or their homologous amino acid sequences are inherent to the full-length sequences and are taught by the  
30 present invention. Such polypeptide fragments preferably are at least 12 amino acids in length. Advantageously, they are at least 15 amino acids, preferably at least 20, 25, 30, 35, 40,

45, 50 amino acids, more preferably at least 55, 60, 65, 70, 75 amino acids, and most preferably at least 80, 85, 90, 95, 100 amino acids in length.

Polynucleotides of 30 to 600 nucleotides encoding  
5 partial sequences of sequences homologous to any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 are retrieved by PCR amplification using the parameters outlined above and using primers matching the sequences upstream and downstream of the 5' and 3' ends of the fragment to be amplified. The template  
10 polynucleotide for such amplification is either the full length polynucleotide homologous to any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, or a polynucleotide contained in a mixture of polynucleotides such as a DNA or RNA library. As an alternative method for retrieving the partial sequences,  
15 screening hybridization is carried out under conditions described above and using the formula for calculating  $T_m$ . Where fragments of 30 to 600 nucleotides are to be retrieved, the calculated  $T_m$  is corrected by subtracting (600/polynucleotide size in base pairs) and the stringency  
20 conditions are defined by a hybridization temperature that is 5 to 10°C below  $T_m$ . Where oligonucleotides shorter than 20-30 bases are to be obtained, the formula for calculating the  $T_m$  is as follows:  $T_m = 4 \times (G+C) + 2 (A+T)$ . For example, an 18 nucleotide fragment of 50% G+C would have an approximate  $T_m$   
25 of 54°C. Short peptides that are fragments of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 or its homologous sequences, are obtained directly by chemical synthesis (E. Gross and H. J. Meinhofer, 4 The Peptides: Analysis, Synthesis, Biology; Modern Techniques of Peptide Synthesis, John Wiley &  
30 Sons (1981), and M. Bodanzki, Principles of Peptide Synthesis, Springer-Verlag (1984)).

Useful polypeptide derivatives, e.g., polypeptide fragments, are designed using computer-assisted analysis of

amino acid sequences. This would identify probable surface-exposed, antigenic regions (Hughes et al., 1992. *Infect. Immun.* 60(9):3497). Analysis of 6 amino acid sequences contained in any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20, based on the product of flexibility and hydrophobicity propensities using the program SEQSEE (Wishart DS, et al. "SEQSEE: a comprehensive program suite for protein sequence analysis." *Comput Appl Biosci.* 1994 Apr;10(2):121-32), reveal potential B- and T-cell epitopes which may be used as a basis for selecting useful immunogenic fragments and variants. This analysis uses a reasonable combination of external surface features that is likely to be recognized by antibodies. Probable T-cell epitopes for HLA-A0201 MHC subclass may be revealed by an algorithms that emulate an approach developed at the NIH (Parker KC, et al. "Peptide binding to MHC class I molecules: implications for antigenic peptide prediction." *Immunol Res* 1995;14(1):34-57). The potential B-cell and T-cell epitopes are shown in Tables 2, 5, 7, 9, 11, 13, 15, 17 and 19 and SEQ ID NOS: 41 to 74. Sequences which are substantially identical to SEQ ID NOS: 41 to 74, or which are conservatively substituted variants of SEQ ID NOS: 41 to 74, are expected to be functional epitopes and are within the scope of the invention.

Epitopes which induce a protective T cell-dependent immune response are present throughout the length of the polypeptide. However, some epitopes may be masked by secondary and tertiary structures of the polypeptide. To reveal such masked epitopes large internal deletions are created which remove much of the original protein structure and exposes the masked epitopes. Such internal deletions sometimes effect the additional advantage of removing immunodominant regions of high variability among strains.

Polynucleotides encoding polypeptide fragments and polypeptides having large internal deletions are constructed using standard methods (Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994). Such methods  
5 include standard PCR, inverse PCR, restriction enzyme treatment of cloned DNA molecules, or the method of Kunkel *et al.* (Kunkel *et al.* Proc. Natl. Acad. Sci. USA (1985) 82:448). Components for these methods and instructions for their use are readily available from various commercial sources such as  
10 Stratagene. Once the deletion mutants have been constructed, they are tested for their ability to prevent or treat *Chlamydia* infection as described above.

As used herein, a fusion polypeptide is one that contains a polypeptide or a polypeptide derivative of the  
15 invention fused at the N- or C-terminal end to any other polypeptide (hereinafter referred to as a peptide tail). A simple way to obtain such a fusion polypeptide is by translation of an in-frame fusion of the polynucleotide sequences, *i.e.*, a hybrid gene. The hybrid gene encoding the  
20 fusion polypeptide is inserted into an expression vector which is used to transform or transfect a host cell. Alternatively, the polynucleotide sequence encoding the polypeptide or polypeptide derivative is inserted into an expression vector in which the polynucleotide encoding the peptide tail is already  
25 present. Such vectors and instructions for their use are commercially available, *e.g.* the pMal-c2 or pMal-p2 system from New England Biolabs, in which the peptide tail is a maltose binding protein, the glutathione-S-transferase system of Pharmacia, or the His-Tag system available from Novagen. These  
30 and other expression systems provide convenient means for further purification of polypeptides and derivatives of the invention.

An advantageous example of a fusion polypeptide is one where the polypeptide or homolog or fragment of the invention is fused to a polypeptide having adjuvant activity, such as subunit B of either cholera toxin or *E. coli* heat-labile toxin. Another advantageous fusion is one where the polypeptide, homolog or fragment is fused to a strong T-cell epitope or B-cell epitope. Such an epitope may be one known in the art (e.g. the Hepatitis B virus core antigen, D.R. Millich *et al.*, "Antibody production to the nucleocapsid and envelope of the Hepatitis B virus primed by a single synthetic T cell site", *Nature*. 1987. 329:547-549), or one which has been identified in another polypeptide of the invention based on computer-assisted analysis of probable T- or B-cell epitopes. Consistent with this aspect of the invention is a fusion polypeptide comprising T- or B-cell epitopes from any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 or its homolog or fragment, wherein the epitopes are derived from multiple variants of said polypeptide or homolog or fragment, each variant differing from another in the location and sequence of its epitope within the polypeptide. Such a fusion is effective in the prevention and treatment of *Chlamydia* infection since it optimizes the T- and B-cell response to the overall polypeptide, homolog or fragment.

To effect fusion, the polypeptide of the invention is fused to the N-, or preferably, to the C-terminal end of the polypeptide having adjuvant activity or T- or B-cell epitope. Alternatively, a polypeptide fragment of the invention is inserted internally within the amino acid sequence of the polypeptide having adjuvant activity. The T- or B-cell epitope may also be inserted internally within the amino acid sequence of the polypeptide of the invention.

Consistent with the first aspect, the polynucleotides of the invention also encode hybrid precursor polypeptides

containing heterologous signal peptides, which mature into polypeptides of the invention. By "heterologous signal peptide" is meant a signal peptide that is not found in naturally-occurring precursors of polypeptides of the invention.

Polynucleotide molecules according to the invention, including RNA, DNA, or modifications or combinations thereof, have various applications. A DNA molecule is used, for example, (i) in a process for producing the encoded polypeptide in a recombinant host system, (ii) in the construction of vaccine vectors such as poxviruses, which are further used in methods and compositions for preventing and/or treating *Chlamydia* infection, (iii) as a vaccine agent (as well as an RNA molecule), in a naked form or formulated with a delivery vehicle and, (iv) in the construction of attenuated *Chlamydia* strains that can over-express a polynucleotide of the invention or express it in a non-toxic, mutated form.

Selected genes from pathogenic micro-organisms within an eukaryotic expression plasmid are useful as vaccines.

Expression plasmids contain methylated CpG motifs that elicit innate cytokine responses that promote the canalization of CD4 T cell responses to a Th1 cytokine secretion pattern. The intracellular synthesis of the microbial protein, especially within transfected professional antigen-presenting cells, facilitates the presentation of antigen on class I and class II molecules and the induction of cell-mediated immunity. The use of one or a number of microbial protein-coding genes allows the presentation of protective antigens to the immune system to occur in the absence of microbe-directed immune evasion mechanisms and in the absence of competing or pathologic antigens. Immune responses primed by DNA vaccines are also readily amplified by protein-antigen immunization. Thus,

immunization with DNA vaccines is particularly relevant to chlamydial vaccine design.

Accordingly, a second aspect of the invention encompasses (i) an expression cassette containing a DNA molecule of the invention placed under the control of the elements required for expression, in particular under the control of an appropriate promoter; (ii) an expression vector containing an expression cassette of the invention; (iii) a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, as well as (iv) a process for producing a polypeptide or polypeptide derivative encoded by a polynucleotide of the invention, which involves culturing a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, under conditions that allow expression of the DNA molecule of the invention and, recovering the encoded polypeptide or polypeptide derivative from the cell culture.

A recombinant expression system is selected from procaryotic and eucaryotic hosts. Eucaryotic hosts include yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris*), mammalian cells (e.g., COS1, NIH3T3, or JEG3 cells), arthropods cells (e.g., *Spodoptera frugiperda* (SF9) cells), and plant cells. A preferred expression system is a procaryotic host such as *E. coli*. Bacterial and eucaryotic cells are available from a number of different sources including commercial sources to those skilled in the art, e.g., the American Type Culture Collection (ATCC; Rockville, Maryland). Commercial sources of cells used for recombinant protein expression also provide instructions for usage of the cells.

The choice of the expression system depends on the features desired for the expressed polypeptide. For example,



it may be useful to produce a polypeptide of the invention in a particular lipidated form or any other form.

One skilled in the art would readily understand that not all vectors and expression control sequences and hosts would be expected to express equally well the polynucleotides of this invention. With the guidelines described below, however, a selection of vectors, expression control sequences and hosts may be made without undue experimentation and without departing from the scope of this invention.

In selecting a vector, the host must be chosen that is compatible with the vector which is to exist and possibly replicate in it. Considerations are made with respect to the vector copy number, the ability to control the copy number, expression of other proteins such as antibiotic resistance. In selecting an expression control sequence, a number of variables are considered. Among the important variable are the relative strength of the sequence (e.g. the ability to drive expression under various conditions), the ability to control the sequence's function, compatibility between the polynucleotide to be expressed and the control sequence (e.g. secondary structures are considered to avoid hairpin structures which prevent efficient transcription). In selecting the host, unicellular hosts are selected which are compatible with the selected vector, tolerant of any possible toxic effects of the expressed product, able to secrete the expressed product efficiently if such is desired, to be able to express the product in the desired conformation, to be easily scaled up, and to which ease of purification of the final product.

The choice of the expression cassette depends on the host system selected as well as the features desired for the expressed polypeptide. Typically, an expression cassette includes a promoter that is functional in the selected host

system and can be constitutive or inducible; a ribosome binding site; a start codon (ATG) if necessary; a region encoding a signal peptide, e.g., a lipidation signal peptide; a DNA molecule of the invention; a stop codon; and optionally a 3' terminal region (translation and/or transcription terminator). The signal peptide encoding region is adjacent to the polynucleotide of the invention and placed in proper reading frame. The signal peptide-encoding region is homologous or heterologous to the DNA molecule encoding the mature polypeptide and is compatible with the secretion apparatus of the host used for expression. The open reading frame constituted by the DNA molecule of the invention, solely or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters and signal peptide encoding regions are widely known and available to those skilled in the art and include, for example, the promoter of *Salmonella typhimurium* (and derivatives) that is inducible by arabinose (promoter *araB*) and is functional in Gram-negative bacteria such as *E. coli* (as described in U.S. Patent No. 5,028,530 and in Cagnon et al., (Cagnon et al., Protein Engineering (1991) 4(7):843)); the promoter of the gene of bacteriophage T7 encoding RNA polymerase, that is functional in a number of *E. coli* strains expressing T7 polymerase (described in U.S. Patent No. 4,952,496); *OspA* lipidation signal peptide; and *RlpB* lipidation signal peptide (Takase et al., J. Bact. (1987) 169:5692).

The expression cassette is typically part of an expression vector, which is selected for its ability to replicate in the chosen expression system. Expression vectors (e.g., plasmids or viral vectors) can be chosen, for example, from those described in Pouwels et al. (Cloning Vectors: A

Laboratory Manual 1985, Supp. 1987). Suitable expression vectors can be purchased from various commercial sources.

Methods for transforming/transfecting host cells with expression vectors are well-known in the art and depend on the  
5 host system selected as described in Ausubel *et al.*, (Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994).

Upon expression, a recombinant polypeptide of the invention (or a polypeptide derivative) is produced and remains  
10 in the intracellular compartment, is secreted/excreted in the extracellular medium or in the periplasmic space, or is embedded in the cellular membrane. The polypeptide is recovered in a substantially purified form from the cell extract or from the supernatant after centrifugation of the  
15 recombinant cell culture. Typically, the recombinant polypeptide is purified by antibody-based affinity purification or by other well-known methods that can be readily adapted by a person skilled in the art, such as fusion of the polynucleotide encoding the polypeptide or its derivative to a small affinity  
20 binding domain. Antibodies useful for purifying by immunoaffinity the polypeptides of the invention are obtained as described below.

A polynucleotide of the invention can also be useful as a vaccine. There are two major routes, either using a viral  
25 or bacterial host as gene delivery vehicle (live vaccine vector) or administering the gene in a free form, e.g., inserted into a plasmid. Therapeutic or prophylactic efficacy of a polynucleotide of the invention is evaluated as described below.

30 Accordingly, a third aspect of the invention provides (i) a vaccine vector such as a poxvirus, containing a DNA molecule of the invention, placed under the control of elements

required for expression; (ii) a composition of matter comprising a vaccine vector of the invention, together with a diluent or carrier; specifically (iii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a vaccine vector of the invention; (iv) a method for inducing an immune response against *Chlamydia* in a mammal (e.g., a human; alternatively, the method can be used in veterinary applications for treating or preventing *Chlamydia* infection of animals, e.g., cats or birds), which involves administering to the mammal an immunogenically effective amount of a vaccine vector of the invention to elicit a protective or therapeutic immune response to *Chlamydia* ; and particularly, (v) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumonia*, *C. pecorum*) infection, which involves administering a prophylactic or therapeutic amount of a vaccine vector of the invention to an infected individual. Additionally, the third aspect of the invention encompasses the use of a vaccine vector of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection.

As used herein, a vaccine vector expresses one or several polypeptides or derivatives of the invention. The vaccine vector may express additionally a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), that enhances the immune response (adjuvant effect). It is understood that each of the components to be expressed is placed under the control of elements required for expression in a mammalian cell.

Consistent with the third aspect of the invention is a composition comprising several vaccine vectors, each of them capable of expressing a polypeptide or derivative of the invention. A composition may also comprise a vaccine vector capable of expressing an additional *Chlamydia* antigen, or a

subunit, fragment, homolog, mutant, or derivative thereof;  
optionally together with or a cytokine such as IL-2 or IL-12.

. A general principle is that recognition of a  
particular antigen is not in itself sufficient to produce an  
5 effective immune response. In some cases, a cell-mediated  
response is appropriate; in others, antibody.

Antigens of microorganisms vary considerably in their  
accessibility to cells of the immune system. Antigens which  
normally occur inside a pathogen may become accessible only  
10 when the pathogen or an infected cell is killed. Even antigens  
expressed at the cell surface may present only a limited range  
of their potential epitopes for antibody binding, depending on  
their orientation in the membrane. Protective structures, such  
as bacterial capsules, further limit the effective recognition  
15 of epitopes.

A distinction should be drawn between the overall  
composition of the immune response, those components of it  
which are important in the resolution of infection and the  
components which are responsible for the prevention of re-  
20 infection. In many cases, particular elements of the immune  
response are critically important; for example, cell-mediated  
immunity in leprosy. Even when considering a particular  
effector system, the response directed against some antigens is  
often much more effective than the responses to others. Immune  
25 responses to particular microbial antigens have different  
degrees of relevance to anti-microbial immunity, depending on  
the nature of the organism, its pathogenicity and the nature of  
the immune response it initiates.

The primary effectors against extracellular pathogens  
30 are antibody and complement. Binding of antibody to receptors  
on the pathogen can prevent it from attaching to its target  
cell. Antibody alone, or more effectively in association with

complement, opsonizes pathogens for uptake by phagocytes expressing Fc receptors and complement receptors CR1 and CR3. Usually this will lead to intracellular destruction of the pathogen but if the phagocyte is unable to destroy it and is a  
5 facultative host cell, then antibody may actually promote the spread of infection. Such an eventuality, however, depends on the dynamic balance between the actions of the humoral and cell-mediated immune responses.

Sometimes effective antibodies must be of the right  
10 class to activate appropriate effectors. The important antigens are those involved in evasion of immune effector mechanisms; that is, pili, fimbriae and capsular antigens which constitute the major antigens of the outer layer of bacteria. Often epitope specificity is important, since it determines  
15 whether complement is deposited in a position to damage the outer membrane. There are also numerous protein antigens which can induce an antibody response; however, although the antibody response is partly species-specific and may be diagnostically useful, it is largely irrelevant to immunity. This is most  
20 obvious in lepromatous leprosy, where the patients have weak cell-mediated immunity, high levels of specific antibody and tissues heavily infected with bacteria.

In some cases, a particular type of antibody response is mandatory for clearance of the pathogen. This is true of  
25 many bacterial infections, where specific antibodies to surface antigens are necessary to neutralize the bacterial defences and opsonize the bacteria for phagocytes.

There are also cases where responses to individual antigens are essential for host immunity. The simplest  
30 examples are the toxins produced by the causative agents of diphtheria, tetanus and clostridial enteritis. The damage produced directly by the infectious agent in these diseases is

slight by comparison with that produced by the secreted toxins. Consequently, protection against these conditions involves immunization to toxoids. Nevertheless, the immune system must still eradicate the primary site of the bacterial infection if  
5 the disease is to be resolved. The target antigens for bactericidal antibodies are extremely diverse and include LPS, capsular polysaccharides and other outer membrane proteins. Virulence factors can also provide good immunogens in a vaccine.

10           Tables 1, 3, 4, 6, 8, 10, 12, 14, 16 and 18, as well as corresponding Figures 31 to 40, demonstrate that the polypeptides disclosed herein are immunogenic. Furthermore, these Figures demonstrate that the polypeptides disclosed herein confer immunoprotection from *Chlamydia* infection, as  
15 evidenced by accelerated clearance of pulmonary infection. Such reduction in the severity of effects of *Chlamydia* infection is evidence that the polypeptides have generated an active functional immune response against the pathogen, rather than a mere antibody response against the antigen.

20           Animal models have been used to define the immunobiologic feature of *C. trachomatis* infection. The mouse model is particularly informative, largely because of the ready availability of immune reagents for murine studies and the development of transgenic and knockout (KO) mice. *C.*  
25 *trachomatis* mouse pneumonitis (MoPn) is the most widely tested biovar among the three *C. trachomatis* biovars (trachoma, lymphogranuloma venereum, and MoPn). Although human biovars have also been used in animal models, they normally require high inocula or pretreatment with progesterone. MoPn, which  
30 was originally isolated from mouse tissues, is thought to be a natural murine pathogen and thus offers an evolutionarily adapted pathogen for analysis of host-pathogen interactions.

The significant progress in chlamydial immunobiology based on murine models of MoPn infection has extended and clarified recent immunoepidemiologic studies in humans (Yang and Brunham (1998) Can J Infect Dis; 9:99-108). In particular, since the discovery of T helper (Th) 1 and 2 subsets, cytokine patterns have been shown to be critical in the regulation of immune responses to a variety of infectious agents including chlamydiae. Clinical investigation has shown that trachoma patients with severe conjunctival scarring have impaired cell-mediated immune responses to *C. trachomatis* and high IgG antibody titers (Yang and Brunham (1999) Curr Opin Infect Dis; 12:47-52). Cytokine analysis shows increased interleukin (IL)-4 and reduced interferon (IFN)- $\gamma$  production in subjects with scarring disease due to *C. trachomatis* infection compared with controls without scarring disease.

Vaccination methods for treating or preventing infection in a mammal comprises use of a vaccine vector of the invention to be administered by any conventional route, particularly to a mucosal (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. Preferred routes depend upon the choice of the vaccine vector. Treatment may be effected in a single dose or repeated at intervals. The appropriate dosage depends on various parameters understood by skilled artisans such as the vaccine vector itself, the route of administration or the condition of the mammal to be vaccinated (weight, age and the like).

Live vaccine vectors available in the art include viral vectors such as adenoviruses and poxviruses as well as bacterial vectors, e.g., *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, Bacille bilié de Calmette-Guérin (BCG), and *Streptococcus*.



An example of an adenovirus vector, as well as a method for constructing an adenovirus vector capable of expressing a DNA molecule of the invention, are described in U.S. Patent No. 4,920,209. Poxvirus vectors include vaccinia and canary pox virus, described in U.S. Patent No. 4,722,848 and U.S. Patent No. 5,364,773, respectively. (Also see, e.g., Tartaglia et al., Virology (1992) 188:217) for a description of a vaccinia virus vector and Taylor et al, Vaccine (1995) 13:539 for a reference of a canary pox.) Poxvirus vectors capable of expressing a polynucleotide of the invention are obtained by homologous recombination as described in Kieny et al., Nature (1984) 312:163 so that the polynucleotide of the invention is inserted in the viral genome under appropriate conditions for expression in mammalian cells. Generally, the dose of vaccine viral vector, for therapeutic or prophylactic use, can be of from about  $1 \times 10^4$  to about  $1 \times 10^{11}$ , advantageously from about  $1 \times 10^7$  to about  $1 \times 10^{10}$ , preferably of from about  $1 \times 10^7$  to about  $1 \times 10^9$  plaque-forming units per kilogram. Preferably, viral vectors are administered parenterally; for example, in 3 doses, 4 weeks apart. It is preferable to avoid adding a chemical adjuvant to a composition containing a viral vector of the invention and thereby minimizing the immune response to the viral vector itself.

Non-toxicogenic *Vibrio cholerae* mutant strains that are useful as a live oral vaccine are known. Mekalanos et al., Nature (1983) 306:551 and U.S. Patent No. 4,882,278 describe strains which have a substantial amount of the coding sequence of each of the two *ctxA* alleles deleted so that no functional *cholerae* toxin is produced. WO 92/11354 describes a strain in which the *irgA* locus is inactivated by mutation; this mutation can be combined in a single strain with *ctxA* mutations. WO 94/01533 describes a deletion mutant lacking functional *ctxA* and *attRS1* DNA sequences. These mutant strains are genetically

engineered to express heterologous antigens, as described in WO 94/19482. An effective vaccine dose of a *Vibrio cholerae* strain capable of expressing a polypeptide or polypeptide derivative encoded by a DNA molecule of the invention contains  
5 about  $1 \times 10^5$  to about  $1 \times 10^9$ , preferably about  $1 \times 10^6$  to about  $1 \times 10^8$ , viable bacteria in a volume appropriate for the selected route of administration. Preferred routes of administration include all mucosal routes; most preferably, these vectors are administered intranasally or orally.

10 Attenuated *Salmonella typhimurium* strains, genetically engineered for recombinant expression of heterologous antigens or not, and their use as oral vaccines are described in Nakayama et al. (Bio/Technology (1988) 6:693) and WO 92/11361. Preferred routes of administration include  
15 all mucosal routes; most preferably, these vectors are administered intranasally or orally.

Other bacterial strains used as vaccine vectors in the context of the present invention are described for *Shigella flexneri* in High et al., EMBO (1992) 11:1991 and Sizemore et  
20 al., Science (1995) 270:299; for *Streptococcus gordonii* in Medaglini et al., Proc. Natl. Acad. Sci. USA (1995) 92:6868; and for Bacille Calmette Guerin in Flynn J.L., Cell. Mol. Biol. (1994) 40 (suppl. I):31, WO 88/06626, WO 90/00594, WO 91/13157, WO 92/01796, and WO 92/21376.

25 In bacterial vectors, the polynucleotide of the invention is inserted into the bacterial genome or remains in a free state as part of a plasmid.

The composition comprising a vaccine bacterial vector of the present invention may further contain an adjuvant. A  
30 number of adjuvants are known to those skilled in the art. Preferred adjuvants are selected as provided below.

Accordingly, a fourth aspect of the invention provides (i) a composition of matter comprising a polynucleotide of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a polynucleotide of the invention; (iii) a method for inducing an immune response against *Chlamydia* in a mammal by administration of an immunogenically effective amount of a polynucleotide of the invention to elicit a protective immune response to *Chlamydia*; and particularly, (iv) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to an infected individual. Additionally, the fourth aspect of the invention encompasses the use of a polynucleotide of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection. A preferred use includes the use of a DNA molecule placed under conditions for expression in a mammalian cell, especially in a plasmid that is unable to replicate in mammalian cells and to substantially integrate in a mammalian genome.

Use of the polynucleotides of the invention include their administration to a mammal as a vaccine, for therapeutic or prophylactic purposes. Such polynucleotides are used in the form of DNA as part of a plasmid that is unable to replicate in a mammalian cell and unable to integrate into the mammalian genome. Typically, such a DNA molecule is placed under the control of a promoter suitable for expression in a mammalian cell. The promoter functions either ubiquitously or tissue-specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (described in U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter (described in Norton & Coffin, *Molec. Cell Biol.* (1985) 5:281).

An example of a tissue-specific promoter is the desmin promoter which drives expression in muscle cells (Li et al., Gene (1989) 78:243, Li & Paulin, J. Biol. Chem. (1991) 266:6562 and Li & Paulin, J. Biol. Chem. (1993) 268:10403). Use of promoters is well-known to those skilled in the art. Useful vectors are described in numerous publications, specifically WO 94/21797 and Hartikka et al., Human Gene Therapy (1996) 7:1205.

Polynucleotides of the invention which are used as vaccines encode either a precursor or a mature form of the corresponding polypeptide. In the precursor form, the signal peptide is either homologous or heterologous. In the latter case, a eucaryotic leader sequence such as the leader sequence of the tissue-type plasminogen factor (tPA) is preferred.

As used herein, a composition of the invention contains one or several polynucleotides with optionally at least one additional polynucleotide encoding another *Chlamydia* antigen such as urease subunit A, B, or both, or a fragment, derivative, mutant, or analog thereof. The composition may also contain an additional polynucleotide encoding a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12) so that the immune response is enhanced. These additional polynucleotides are placed under appropriate control for expression. Advantageously, DNA molecules of the invention and/or additional DNA molecules to be included in the same composition, are present in the same plasmid.

Standard techniques of molecular biology for preparing and purifying polynucleotides are used in the preparation of polynucleotide therapeutics of the invention. For use as a vaccine, a polynucleotide of the invention is formulated according to various methods outlined below.

One method utilizes the polynucleotide in a naked form, free of any delivery vehicles. Such a polynucleotide is

simply diluted in a physiologically acceptable solution such as sterile saline or sterile buffered saline, with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength, such as provided by a sucrose solution, e.g., a solution containing 20% sucrose.

An alternative method utilizes the polynucleotide in association with agents that assist in cellular uptake. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine (see, e.g., WO 94/16737), (ii) liposomes for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides.

Anionic and neutral liposomes are well-known in the art (see, e.g., Liposomes: A Practical Approach, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides.

Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin™ also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis(oleyloxy)-3-(trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidoglycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a

neutral lipid such as DOPE (dioleoyl phosphatidylethanolamine), as described in WO 90/11092 as an example.

Formulation containing cationic liposomes may optionally contain other transfection-facilitating compounds. 5 A number of them are described in WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/02397. They include spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine S, and 10 cationic bile salts (see, for example, WO 93/19768).

Gold or tungsten microparticles are used for gene delivery, as described in WO 91/00359, WO 93/17706, and Tang et al. Nature (1992) 356:152. The microparticle-coated polynucleotide is injected via intradermal or intraepidermal 15 routes using a needleless injection device ("gene gun"), such as those described in U.S. Patent No. 4,945,050, U.S. Patent No. 5,015,580, and WO 94/24263.

The amount of DNA to be used in a vaccine recipient depends, e.g., on the strength of the promoter used in the DNA 20 construct, the immunogenicity of the expressed gene product, the condition of the mammal intended for administration (e.g., the weight, age, and general health of the mammal), the mode of administration, and the type of formulation. In general, a therapeutically or prophylactically effective dose from about 25 1 µg to about 1 mg, preferably, from about 10 µg to about 800 µg and, more preferably, from about 25 µg to about 250 µg, can be administered to human adults. The administration can be achieved in a single dose or repeated at intervals.

The route of administration is any conventional route 30 used in the vaccine field. As general guidance, a polynucleotide of the invention is administered via a mucosal surface, e.g., an ocular, intranasal, pulmonary, oral,

intestinal, rectal, vaginal, and urinary tract surface; or via a parenteral route, e.g., by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route. The choice of administration route depends on the  
5 formulation that is selected. A polynucleotide formulated in association with bupivacaine is advantageously administered into muscles. When a neutral or anionic liposome or a cationic lipid, such as DOTMA or DC-Chol, is used, the formulation can be advantageously injected *via* intravenous, intranasal  
10 (aerosolization), intramuscular, intradermal, and subcutaneous routes. A polynucleotide in a naked form can advantageously be administered *via* the intramuscular, intradermal, or subcutaneous routes.

Although not absolutely required, such a composition  
15 can also contain an adjuvant. If so, a systemic adjuvant that does not require concomitant administration in order to exhibit an adjuvant effect is preferable such as, e.g., QS21, which is described in U.S. Patent No. 5,057,546.

The sequence information provided in the present  
20 application enables the design of specific nucleotide probes and primers that are used for diagnostic purposes. Accordingly, a fifth aspect of the invention provides a nucleotide probe or primer having a sequence found in or derived by degeneracy of the genetic code from a sequence shown  
25 in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19.

The term "probe" as used in the present application refers to DNA (preferably single stranded) or RNA molecules (or modifications or combinations thereof) that hybridize under the stringent conditions, as defined above, to nucleic acid  
30 molecules having any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 or to a sequence homologous to any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, or to its

complementary or anti-sense sequence. Generally, probes are significantly shorter than full-length sequences. Such probes contain from about 5 to about 100, preferably from about 10 to about 80, nucleotides. In particular, probes have sequences

5 that are at least 75%, preferably at least 80% or 85%, more preferably 90% or 95% homologous to a portion of any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 or that are complementary to such sequences. Probes may contain modified bases such as inosine, methyl-5-deoxycytidine, deoxyuridine,

10 dimethylamino-5-deoxyuridine, or diamino-2, 6-purine. Sugar or phosphate residues may also be modified or substituted. For example, a deoxyribose residue may be replaced by a polyamide (Nielsen et al., Science (1991) 254:1497) and phosphate residues may be replaced by ester groups such as diphosphate,

15 alkyl, arylphosphonate and phosphorothioate esters. In addition, the 2'-hydroxyl group on ribonucleotides may be modified by including such groups as alkyl groups.

Probes of the invention are used in diagnostic tests, as capture or detection probes. Such capture probes are

20 conventionally immobilized on a solid support, directly or indirectly, by covalent means or by passive adsorption. A detection probe is labelled by a detection marker selected from: radioactive isotopes, enzymes such as peroxidase, alkaline phosphatase, and enzymes able to hydrolyze a

25 chromogenic, fluorogenic, or luminescent substrate, compounds that are chromogenic, fluorogenic, or luminescent, nucleotide base analogs, and biotin.

Probes of the invention are used in any conventional hybridization technique, such as dot blot (Maniatis et al.,

30 Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), Southern blot (Southern, J. Mol. Biol. (1975) 98:503), northern blot (identical to Southern blot with the exception that RNA is



used as a target), or the sandwich technique (Dunn et al., Cell (1977) 12:23). The latter technique involves the use of a specific capture probe and/or a specific detection probe with nucleotide sequences that at least partially differ from each other.

A primer is a probe of usually about 10 to about 40 nucleotides that is used to initiate enzymatic polymerization of DNA in an amplification process (e.g., PCR), in an elongation process, or in a reverse transcription method. Primers used in diagnostic methods involving PCR are labeled by methods known in the art.

As described herein, the invention also encompasses (i) a reagent comprising a probe of the invention for detecting and/or identifying the presence of *Chlamydia* in a biological material; (ii) a method for detecting and/or identifying the presence of *Chlamydia* in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA or RNA is extracted from the material and denatured, and (c) exposed to a probe of the invention, for example, a capture, detection probe or both, under stringent hybridization conditions, such that hybridization is detected; and (iii) a method for detecting and/or identifying the presence of *Chlamydia* in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA is extracted therefrom, (c) the extracted DNA is primed with at least one, and preferably two, primers of the invention and amplified by polymerase chain reaction, and (d) the amplified DNA fragment is produced.

It is apparent that disclosure of a polynucleotide sequence of any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, its homologs and partial sequences enable their corresponding amino acid sequences. Accordingly, a sixth

aspect of the invention features a substantially purified polypeptide or polypeptide derivative having an amino acid sequence encoded by a polynucleotide of the invention.

A "substantially purified polypeptide" as used herein is defined as a polypeptide that is separated from the environment in which it naturally occurs and/or that is free of the majority of the polypeptides that are present in the environment in which it was synthesized. For example, a substantially purified polypeptide is free from cytoplasmic polypeptides. Those skilled in the art would readily understand that the polypeptides of the invention may be purified from a natural source, *i.e.*, a *Chlamydia* strain, or produced by recombinant means.

Consistent with the sixth aspect of the invention are polypeptides, homologs or fragments which are modified or treated to enhance their immunogenicity in the target animal, in whom the polypeptide, homolog or fragments are intended to confer protection against *Chlamydia*. Such modifications or treatments include: amino acid substitutions with an amino acid derivative such as 3-methylhistidine, 4-hydroxyproline, 5-hydroxylysine etc., modifications or deletions which are carried out after preparation of the polypeptide, homolog or fragment, such as the modification of free amino, carboxyl or hydroxyl side groups of the amino acids.

Identification of homologous polypeptides or polypeptide derivatives encoded by polynucleotides of the invention which have specific antigenicity is achieved by screening for cross-reactivity with an antiserum raised against the polypeptide of reference having an amino acid sequence of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. The procedure is as follows: a monospecific hyperimmune antiserum is raised against a purified reference polypeptide, a

fusion polypeptide (for example, an expression product of MBP, GST, or His-tag systems, the description and instructions for use of which are contained in Invitrogen product manuals for pcDNA3.1/Myc-His(+) A, B, and C and for the Xpress<sup>™</sup> System Protein Purification), or a synthetic peptide predicted to be antigenic. Where an antiserum is raised against a fusion polypeptide, two different fusion systems are employed. Specific antigenicity can be determined according to a number of methods, including Western blot (Towbin et al., Proc. Natl. Acad. Sci. USA (1979) 76:4350), dot blot, and ELISA, as described below.

In a Western blot assay, the product to be screened, either as a purified preparation or a total *E. coli* extract, is submitted to SDS-Page electrophoresis as described by Laemmli (Nature (1970) 227:680). After transfer to a nitrocellulose membrane, the material is further incubated with the monospecific hyperimmune antiserum diluted in the range of dilutions from about 1:5 to about 1:5000, preferably from about 1:100 to about 1:500. Specific antigenicity is shown once a band corresponding to the product exhibits reactivity at any of the dilutions in the above range.

In an ELISA assay, the product to be screened is preferably used as the coating antigen. A purified preparation is preferred, although a whole cell extract can also be used. Briefly, about 100  $\mu$ l of a preparation at about 10  $\mu$ g protein/ml are distributed into wells of a 96-well polycarbonate ELISA plate. The plate is incubated for 2 hours at 37°C then overnight at 4°C. The plate is washed with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBS/Tween buffer). The wells are saturated with 250  $\mu$ l PBS containing 1% bovine serum albumin (BSA) to prevent non-specific antibody binding. After 1 hour incubation at 37°C, the plate is washed with PBS/Tween buffer. The antiserum is

- serially diluted in PBS/Tween buffer containing 0.5% BSA. 100  $\mu$ l of dilutions are added per well. The plate is incubated for 90 minutes at 37°C, washed and evaluated according to standard procedures. For example, a goat anti-rabbit peroxidase
- 5 conjugate is added to the wells when specific antibodies were raised in rabbits. Incubation is carried out for 90 minutes at 37°C and the plate is washed. The reaction is developed with the appropriate substrate and the reaction is measured by colorimetry (absorbance measured spectrophotometrically).
- 10 Under the above experimental conditions, a positive reaction is shown by O.D. values greater than a non immune control serum.

- In a dot blot assay, a purified product is preferred, although a whole cell extract can also be used. Briefly, a solution of the product at about 100  $\mu$ g/ml is serially two-fold
- 15 diluted in 50 mM Tris-HCl (pH 7.5). 100  $\mu$ l of each dilution are applied to a nitrocellulose membrane 0.45  $\mu$ m set in a 96-well dot blot apparatus (Biorad). The buffer is removed by applying vacuum to the system. Wells are washed by addition of 50 mM Tris-HCl (pH 7.5) and the membrane is air-dried. The
- 20 membrane is saturated in blocking buffer (50 mM Tris-HCl (pH 7.5) 0.15 M NaCl, 10 g/L skim milk) and incubated with an antiserum dilution from about 1:50 to about 1:5000, preferably about 1:500. The reaction is revealed according to standard procedures. For example, a goat anti-rabbit peroxidase
- 25 conjugate is added to the wells when rabbit antibodies are used. Incubation is carried out 90 minutes at 37°C and the blot is washed. The reaction is developed with the appropriate substrate and stopped. The reaction is measured visually by the appearance of a colored spot, e.g., by colorimetry. Under
- 30 the above experimental conditions, a positive reaction is shown once a colored spot is associated with a dilution of at least about 1:5, preferably of at least about 1:500.

Therapeutic or prophylactic efficacy of a polypeptide or derivative of the invention can be evaluated as described below. A seventh aspect of the invention provides (i) a composition of matter comprising a polypeptide of the invention  
5 together with a diluent or carrier; specifically (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a polypeptide of the invention; (iii) a method for inducing an immune response against *Chlamydia* in a mammal, by administering to the mammal  
10 an immunogenically effective amount of a polypeptide of the invention to elicit a protective immune response to *Chlamydia*; and particularly, (iv) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or  
15 therapeutic amount of a polypeptide of the invention to an infected individual. Additionally, the seventh aspect of the invention encompasses the use of a polypeptide of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection.

20 As used herein, the immunogenic compositions of the invention are administered by conventional routes known the vaccine field, in particular to a mucosal (e.g., ocular, intranasal, pulmonary, oral, gastric, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g.,  
25 subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. The choice of administration route depends upon a number of parameters, such as the adjuvant associated with the polypeptide. If a mucosal adjuvant is used, the intranasal or oral route is preferred. If a lipid  
30 formulation or an aluminum compound is used, the parenteral route is preferred with the sub-cutaneous or intramuscular route being most preferred. The choice also depends upon the nature of the vaccine agent. For example, a polypeptide of the

invention fused to CTB or LTB is best administered to a mucosal surface.

As used herein, the composition of the invention contains one or several polypeptides or derivatives of the invention. The composition optionally contains at least one additional *Chlamydia* antigen, or a subunit, fragment, homolog, mutant, or derivative thereof.

For use in a composition of the invention, a polypeptide or derivative thereof is formulated into or with liposomes, preferably neutral or anionic liposomes, microspheres, ISCOMS, or virus-like-particles (VLPs) to facilitate delivery and/or enhance the immune response. These compounds are readily available to one skilled in the art; for example, see Liposomes: A Practical Approach, RCP New Ed, IRL press (1990).

Adjuvants other than liposomes and the like are also used and are known in the art. Adjuvants may protect the antigen from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. An appropriate selection can conventionally be made by those skilled in the art, for example, from those described below (under the eleventh aspect of the invention).

Treatment is achieved in a single dose or repeated as necessary at intervals, as can be determined readily by one skilled in the art. For example, a priming dose is followed by three booster doses at weekly or monthly intervals. An appropriate dose depends on various parameters including the recipient (e.g., adult or infant), the particular vaccine antigen, the route and frequency of administration, the presence/absence or type of adjuvant, and the desired effect

(e.g., protection and/or treatment), as can be determined by one skilled in the art. In general, a vaccine antigen of the invention is administered by a mucosal route in an amount from about 10  $\mu$ g to about 500 mg, preferably from about 1 mg to about 200 mg. For the parenteral route of administration, the dose usually does not exceed about 1 mg, preferably about 100  $\mu$ g.

When used as vaccine agents, polynucleotides and polypeptides of the invention may be used sequentially as part of a multistep immunization process. For example, a mammal is initially primed with a vaccine vector of the invention such as a pox virus, e.g., via the parenteral route, and then boosted twice with the polypeptide encoded by the vaccine vector, e.g., via the mucosal route. In another example, liposomes associated with a polypeptide or derivative of the invention is also used for priming, with boosting being carried out mucosally using a soluble polypeptide or derivative of the invention in combination with a mucosal adjuvant (e.g., LT).

A polypeptide derivative of the invention is also used in accordance with the seventh aspect as a diagnostic reagent for detecting the presence of anti-*Chlamydia* antibodies, e.g., in a blood sample. Such polypeptides are about 5 to about 80, preferably about 10 to about 50 amino acids in length. They are either labeled or unlabeled, depending upon the diagnostic method. Diagnostic methods involving such a reagent are described below.

Upon expression of a DNA molecule of the invention, a polypeptide or polypeptide derivative is produced and purified using known laboratory techniques. As described above, the polypeptide or polypeptide derivative may be produced as a fusion protein containing a fused tail that facilitates purification. The fusion product is used to immunize a small

mammal, e.g., a mouse or a rabbit, in order to raise antibodies against the polypeptide or polypeptide derivative (monospecific antibodies). Accordingly, an eighth aspect of the invention provides a monospecific antibody that binds to a polypeptide or  
5 polypeptide derivative of the invention.

By "monospecific antibody" is meant an antibody that is capable of reacting with a unique naturally-occurring *Chlamydia* polypeptide. An antibody of the invention is either polyclonal or monoclonal. Monospecific antibodies may be  
10 recombinant, e.g., chimeric (e.g., constituted by a variable region of murine origin associated with a human constant region), humanized (a human immunoglobulin constant backbone together with hypervariable region of animal, e.g., murine, origin), and/or single chain. Both polyclonal and monospecific  
15 antibodies may also be in the form of immunoglobulin fragments, e.g., F(ab)'2 or Fab fragments. The antibodies of the invention are of any isotype, e.g., IgG or IgA, and polyclonal antibodies are of a single isotype or a mixture of isotypes.

Antibodies against the polypeptides, homologs or  
20 fragments of the present invention are generated by immunization of a mammal with a composition comprising said polypeptide, homolog or fragment. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal or monoclonal antibodies are well known in the art. For a review,  
25 see "Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Eds. E. Harlow and D. Lane (1988), and D.E. Yelton et al., 1981. Ann. Rev. Biochem. 50:657-680. For monoclonal antibodies, see Kohler & Milstein (1975) Nature 256:495-497.

The antibodies of the invention, which are raised to  
30 a polypeptide or polypeptide derivative of the invention, are produced and identified using standard immunological assays, e.g., Western blot analysis, dot blot assay, or ELISA (see,



e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY). The antibodies are used in diagnostic methods to detect the presence of a *Chlamydia* antigen in a sample, such as a biological sample.

5 The antibodies are also used in affinity chromatography for purifying a polypeptide or polypeptide derivative of the invention. As is discussed further below, such antibodies may be used in prophylactic and therapeutic passive immunization methods.

10 Accordingly, a ninth aspect of the invention provides (i) a reagent for detecting the presence of *Chlamydia* in a biological sample that contains an antibody, polypeptide, or polypeptide derivative of the invention; and (ii) a diagnostic method for detecting the presence of *Chlamydia* in a biological  
15 sample, by contacting the biological sample with an antibody, a polypeptide, or a polypeptide derivative of the invention, such that an immune complex is formed, and by detecting such complex to indicate the presence of *Chlamydia* in the sample or the organism from which the sample is derived.

20 Those skilled in the art will readily understand that the immune complex is formed between a component of the sample and the antibody, polypeptide, or polypeptide derivative, whichever is used, and that any unbound material is removed prior to detecting the complex. It is understood that a  
25 polypeptide reagent is useful for detecting the presence of anti-*Chlamydia* antibodies in a sample, e.g., a blood sample, while an antibody of the invention is used for screening a sample, such as a gastric extract or biopsy, for the presence of *Chlamydia* polypeptides.

30 For diagnostic applications, the reagent (i.e., the antibody, polypeptide, or polypeptide derivative of the invention) is either in a free state or immobilized on a solid

support, such as a tube, a bead, or any other conventional support used in the field. Immobilization is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between  
5 the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support. For example, if a polypeptide reagent is used, an antibody that binds to it can serve as an anti-reagent, provided that it binds to an epitope that is not  
10 involved in the recognition of antibodies in biological samples. Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the polypeptide reagent and the corresponding receptor immobilized on the solid phase. This is illustrated  
15 by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

Such diagnostic agents may be included in a kit which  
20 also comprises instructions for use. The reagent is labeled with a detection means which allows for the detection of the reagent when it is bound to its target. The detection means may be a fluorescent agent such as fluorescein isocyanate or fluorescein isothiocyanate, or an enzyme such as horse radish  
25 peroxidase or luciferase or alkaline phosphatase, or a radioactive element such as  $^{125}\text{I}$  or  $^{51}\text{Cr}$ .

Accordingly, a tenth aspect of the invention provides a process for purifying, from a biological sample, a polypeptide or polypeptide derivative of the invention, which  
30 involves carrying out antibody-based affinity chromatography with the biological sample, wherein the antibody is a monospecific antibody of the invention.

For use in a purification process of the invention, the antibody is either polyclonal or monospecific, and preferably is of the IgG type. Purified IgGs is prepared from an antiserum using standard methods (see, e.g., Coligan et al.,  
5 Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY.). Conventional chromatography supports, as well as standard methods for grafting antibodies, are described in, e.g., Antibodies: A Laboratory Manual, D. Lane, E. Harlow, Eds. (1988) and outlined below.

10 Briefly, a biological sample, such as an *C. pneumoniae* extract preferably in a buffer solution, is applied to a chromatography material, preferably equilibrated with the buffer used to dilute the biological sample so that the polypeptide or polypeptide derivative of the invention (i.e.,  
15 the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to an antibody of the invention, is in either a batch form or a column. The unbound components are washed off and the antigen is then eluted with an appropriate elution buffer, such as a  
20 glycine buffer or a buffer containing a chaotropic agent, e.g., guanidine HCl, or high salt concentration (e.g., 3 M MgCl<sub>2</sub>). Eluted fractions are recovered and the presence of the antigen is detected, e.g., by measuring the absorbance at 280 nm.

An eleventh aspect of the invention provides (i) a  
25 composition of matter comprising a monospecific antibody of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a monospecific antibody of the invention, and (iii) a method for treating or preventing a  
30 *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae* or *C. pecorum*) infection, by administering a therapeutic or prophylactic amount of a monospecific antibody of the invention to an infected individual. Additionally, the eleventh aspect

of the invention encompasses the use of a monospecific antibody of the invention in the preparation of a medicament for treating or preventing *Chlamydia* infection.

The monospecific antibody is either polyclonal or  
5 monoclonal, preferably of the IgA isotype (predominantly). In passive immunization, the antibody is administered to a mucosal surface of a mammal, e.g., the gastric mucosa, e.g., orally or intragastrically, advantageously, in the presence of a bicarbonate buffer. Alternatively, systemic administration,  
10 not requiring a bicarbonate buffer, is carried out. A monospecific antibody of the invention is administered as a single active component or as a mixture with at least one monospecific antibody specific for a different *Chlamydia* polypeptide. The amount of antibody and the particular regimen  
15 used are readily determined by one skilled in the art. For example, daily administration of about 100 to 1,000 mg of antibodies over one week, or three doses per day of about 100 to 1,000 mg of antibodies over two or three days, are effective regimens for most purposes.

20 Therapeutic or prophylactic efficacy are evaluated using standard methods in the art, e.g., by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity, using, e.g., the *C. pneumoniae* mouse model. Those skilled in the art will readily recognize that  
25 the *C. pneumoniae* strain of the model may be replaced with another *Chlamydia* strain. For example, the efficacy of DNA molecules and polypeptides from *C. pneumoniae* is preferably evaluated in a mouse model using *C. pneumoniae* strain. Protection is determined by comparing the degree of *Chlamydia*  
30 infection to that of a control group. Protection is shown when infection is reduced by comparison to the control group. Such an evaluation is made for polynucleotides, vaccine vectors,

polypeptides and derivatives thereof, as well as antibodies of the invention.

Adjuvants useful in any of the vaccine compositions described above are as follows.

- 5           Adjuvants for parenteral administration include aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen is precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants, such as RIBI  
10 (ImmunoChem, Hamilton, MT), are used in parenteral administration.

- Adjuvants for mucosal administration include bacterial toxins, e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostridium difficile* toxin A and  
15 the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof such as a purified preparation of native cholera toxin subunit B (CTB). Fragments, homologs, derivatives, and fusions to any of these toxins are also suitable, provided that they retain adjuvant activity.  
20 Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/06627 (Arg-192-Gly LT mutant), and WO 95/34323 (Arg-9-Lys and Glu-129-Gly PT mutant). Additional LT mutants that are used in the methods and compositions of the invention  
25 include, e.g., Ser-63-Lys, Ala-69Gly, Glu-110-Asp, and Glu-112-Asp mutants. Other adjuvants, such as a bacterial monophosphoryl lipid A (MPLA) of, e.g., *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*; saponins, or polylactide glycolide (PLGA) microspheres, is also  
30 be used in mucosal administration.

Adjuvants useful for both mucosal and parenteral administrations include polyphosphazene (WO 95/02415), DC-chol

(3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol;  
U.S. Patent No. 5,283,185 and WO 96/14831) and QS-21  
(WO 88/09336).

Any pharmaceutical composition of the invention  
5 containing a polynucleotide, a polypeptide, a polypeptide  
derivative, or an antibody of the invention, is manufactured in  
a conventional manner. In particular, it is formulated with a  
pharmaceutically acceptable diluent or carrier, e.g., water or  
a saline solution such as phosphate buffer saline. In general,  
10 a diluent or carrier is selected on the basis of the mode and  
route of administration, and standard pharmaceutical practice.  
Suitable pharmaceutical carriers or diluents, as well as  
pharmaceutical necessities for their use in pharmaceutical  
formulations, are described in *Remington's Pharmaceutical*  
15 *Sciences*, a standard reference text in this field and in the  
USP/NF.

The invention also includes methods in which  
*Chlamydia* infection are treated by oral administration of a  
*Chlamydia* polypeptide of the invention and a mucosal adjuvant,  
20 in combination with an antibiotic, an antacid, sucralfate, or a  
combination thereof. Examples of such compounds that can be  
administered with the vaccine antigen and the adjuvant are  
antibiotics, including, e.g., macrolides, tetracyclines, and  
derivatives thereof (specific examples of antibiotics that can  
25 be used include azithromycin or doxycycline or immunomodulators  
such as cytokines or steroids). In addition, compounds  
containing more than one of the above-listed components coupled  
together, are used. The invention also includes compositions  
for carrying out these methods, i.e., compositions containing a  
30 *Chlamydia* antigen (or antigens) of the invention, an adjuvant,  
and one or more of the above-listed compounds, in a  
pharmaceutically acceptable carrier or diluent.

It has recently been shown that the 60kDa cysteine rich membrane protein contains a sequence cross-reactive with the murine alpha-myosin heavy chain epitope M7A-alpha, an epitope conserved in humans (Bachmaier *et al.*, Science (1999) 283:1335). This cross-reactivity is proposed to contribute to the development of cardiovascular disease, so it may be beneficial to remove this epitope, and any other epitopes cross-reactive with human antigens, from the protein if it is to be used as a vaccine. Accordingly, a further embodiment of the present invention includes the modification of the coding sequence, for example, by deletion or substitution of the nucleotides encoding the epitope from polynucleotides encoding the protein, as to improve the efficacy and safety of the protein as a vaccine. A similar approach may be appropriate for any protective antigen found to have unwanted homologies or cross-reactivities with human antigens.

Amounts of the above-listed compounds used in the methods and compositions of the invention are readily determined by one skilled in the art. Treatment/immunization schedules are also known and readily designed by one skilled in the art. For example, the non-vaccine components can be administered on days 1-14, and the vaccine antigen + adjuvant can be administered on days 7, 14, 21, and 28.

#### **EXAMPLES**

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are

intended in a descriptive sense and not for purposes of limitation.

**Example 1:**

These examples illustrate the preparation of plasmid vectors used in immunoprotection studies.

**A. Preparation of plasmid vector pCACPNM213**

The ATP-binding cassette gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWLO29 by polymerase chain reaction (PCR) using a 5' primer  
10 (5' ATAAGAATGCGGCCGCCACCATGAAGATGCATAGGCTTAAACC 3'; SEQ ID No:21) and a 3' primer  
(5' GCGCCGGATCCACTTAAGATATCGATATTTTGGAG 3'; SEQ ID No:22).  
The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end  
15 of the ATP-binding cassette protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the ATP-binding cassette protein gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the  
20 Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 21) with  
25 transcription under control of the human CMV promoter.

**B. Preparation of plasmid vector pCACPNM882**

The secretory locus ORF gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWLO29 by polymerase chain reaction (PCR) using a 5' primer  
30 (5' ATAAGAATGCGGCCGCCACCATGCGGTTGGGAATAAGCCTATGC 3'; SEQ ID



No:23) and a 3' primer

(5' GCGCCGGTACCGTAATTTAATACTCTTTGAAGGGC 3'; SEQ ID No:24). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the secretory locus ORF coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the secretory locus ORF protein and a KpnI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and KpnI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 22) with transcription under control of the human CMV promoter.

#### C. Preparation of plasmid vector pCACPNM208

The endopeptidase gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWLO29 by polymerase chain reaction (PCR) using a 5' primer

(5' ATAAGAATGCGGCCGCCACCATGCTCACCCCTAGGCTTGGAAAGTTCTTG 3'; SEQ

ID No:25) and a 3' primer

(5' GCTTTGGAGGATCCCGGAGAGGCTAAGGAGAATGG 3'; SEQ ID No:26).

The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the endopeptidase protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the endopeptidase protein gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic

expression vector described in Example 2 (Figure 23) with transcription under control of the human CMV promoter.

#### D. Preparation of plasmid vector pCACPNM1096

The protease gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGAAAAAGGGAAATTAGGAGCC 3'; SEQ ID No:27) and a 3' primer (5' GCGCCGGATCCCGAAGCAGAAGTCGTTGTGGG 3'; SEQ ID No:28). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the protease protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the protease protein gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 24) with transcription under control of the human CMV promoter.

#### E. Preparation of plasmid vector pCACPNM1097

The metalloprotease gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGAGAAAACCTTATTTTATGCAATCCTA 3'; SEQ ID No:29) and a 3' primer (5' GCGCCGGATCCCGAACAACGGAGTCTCTTTGG 3'; SEQ ID No:30). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the metalloprotease protein coding sequence. The 3' primer

includes the sequence encoding the C-terminal sequence of the metalloprotease protein gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

- 5           After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 25) with transcription under control of the human CMV promoter.

10    F. Preparation of plasmid vector pCACPNM908

- The CLP protease ATPase gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGAATAAAAAAATCTAACTATTTG 3'; SEQ ID No:31) and a 3' primer (5' GCGCCGGATCCCAGCGATAGCTTCTGGGGTCC 3'; SEQ ID No:32). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the CLP protease ATPase protein coding sequence. The 3' primer includes the sequence encoding the C-terminal
- 15           sequence of the CLP protease ATPase gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

- After amplification, the PCR fragment was purified
- 25    using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 26) with transcription under control of the human CMV promoter.

#### G. Preparation of plasmid vector pCACPNM909

The gene encoding CLP protease subunit was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer  
5 (5' ATAAGAATGCGGCCGCCACCATGACACTGGTACCCATGTTG 3'; SEQ ID No:33) and a 3' primer  
(5' GCGCCGGATCCCACTGCTACTTGTATCCTTATTAG 3'; SEQ ID No:34). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the  
10 CLP protease subunit coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the CLP protease subunit gene and a BamHI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

- 15 After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 27) with transcription under control of the human CMV promoter.

#### 20 H. Preparation of plasmid vector pCACPNM440

The translycolase / transpeptidase gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer

- (5' ATAAGAATGCGGCCGCCACCATGAGCTACCGTAAACGTTCCGACTC 3'; SEQ ID  
25 No:35) and a 3' primer  
(5' GCGCCGGATCCCACTCGTTCCCCCTTGTTCGGAG 3'; SEQ ID No:36). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the translycolase / transpeptidase coding sequence. The 3' primer  
30 includes the sequence encoding the C-terminal sequence of the translycolase / transpeptidase gene and a BamHI restriction

site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and BamHI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 28) with transcription under control of the human CMV promoter.

#### I. Preparation of plasmid vector pCACPNM459

The gene encoding CLPc protease was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCGCCACCATGTTTGAGAAGTTCACATAAGAGC 3'; SEQ ID No:37) and a 3' primer (5' GCGCCGGTACCGTGATTCCAAGTGAGGCTAGGG 3'; SEQ ID No:38). The 5' primer contains a NotI restriction site, a ribosome binding site, an initiation codon and a sequence at the 5' end of the CLPc protease coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the CLPc protease gene and a KpnI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen), digested with NotI and KpnI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 2 (Figure 29) with transcription under control of the human CMV promoter.

#### J. Preparation of plasmid vector pCACPNM708

The thioredoxin gene was amplified from *Chlamydia pneumoniae* genomic DNA strain CWL029 by polymerase chain

reaction (PCR) using a 5' primer  
(5' ATAAGAATGCGGCCGCCACCATGGTAAAGATCATATCAAGTG 3'; SEQ ID  
No:39) and a 3' primer (5' GCGCCGGATCCCAGCGTGCTTATTGATAAG 3';  
SEQ ID No:40). The 5' primer contains a NotI restriction site,  
5 a ribosome binding site, an initiation codon and a sequence at  
the 5' end of the thioredoxin coding sequence. The 3' primer  
includes the sequence encoding the C-terminal sequence of the  
thioredoxin gene and a BamHI restriction site. The stop codon  
was excluded and an additional nucleotide was inserted to  
10 obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified  
using QIAquick™ PCR purification kit (Qiagen), digested with  
NotI and BamHI and cloned into the pCA-Myc-His eukaryotic  
expression vector described in Example 2 (Figure 30) with  
15 transcription under control of the human CMV promoter.

#### Example 2:

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was  
restricted with SpeI and BamHI to remove the CMV promoter and  
the remaining vector fragment was isolated. The CMV promoter  
20 and intron A from plasmid VR-1012 (Vical) was isolated on a  
SpeI / BamHI fragment. The fragments were ligated together to  
produce plasmid pCA/Myc-His.

The NotI/BamHI restricted PCR fragment containing the  
ATP-binding cassette gene was ligated into the NotI and BamHI  
25 restricted plasmid pCA/Myc-His to produce plasmid pCACPNM213  
(Figure 21).

The NotI/KpnI restricted PCR fragment containing the  
Secretory locus ORF gene was ligated into the NotI and KpnI  
restricted plasmid pCA/Myc-His to produce plasmid pCACPNM882  
30 (Figure 22).

The NotI/BamHI restricted PCR fragment containing the endopeptidase gene was ligated into the NotI and BamHI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM208 (Figure 23).

5           The NotI/BamHI restricted PCR fragment containing the Protease gene was ligated into the NotI and BamHI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM1096 (Figure 24).

          The NotI/BamHI restricted PCR fragment containing the Metalloprotease gene was ligated into the NotI and BamHI  
10 restricted plasmid pCA/Myc-His to produce plasmid pCACPMM1097 (Figure 25).

          The NotI/BamHI restricted PCR fragment containing the CLP protease ATPase gene was ligated into the NotI and BamHI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM908  
15 (Figure 26).

          The NotI/BamHI restricted PCR fragment containing the CLP protease subunit gene was ligated into the NotI and BamHI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM909 (Figure 27).

20           The NotI/BamHI restricted PCR fragment containing the transglycolase/transpeptidase gene was ligated into the NotI and BamHI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM440 (Figure 28).

          The NotI/KpnI restricted PCR fragment containing the  
25 CLPc protease gene was ligated into the NotI and KpnI restricted plasmid pCA/Myc-His to produce plasmid pCACPMM459 (Figure 29).

          The NotI/BamHI restricted PCR fragment containing the Thioredoxin gene was ligated into the NotI and BamHI restricted  
30 plasmid pCA/Myc-His to produce plasmid pCACPMM708 (Figure 30).

Each of the resulting plasmids pCACPNM213, pCACPNM882, pCACPNM208, pCACPNM1096, pCACPNM1097, pCACPNM909, pCACPNM440, pCACPNM459 and pCACPNM708, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which was

5 grown in LB broth containing 50 µg/ml carbenicillin. The plasmid was isolated by the Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and ethidium

10 bromide staining by comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 3:

15 This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*.

It has been previously demonstrated (Yang *et al.* Infect. Immun. May 1993. 61(5):2037-40) that mice are

20 susceptible to intranasal infection with different isolates of *C. pneumoniae*. Strain AR-39 (Grayston *et al* (1990) Journal of Infectious Diseases 161:618-625) was used in Balb/c mice as a challenge infection model to examine the capacity of *Chlamydia* gene products delivered as naked DNA to elicit a protective

25 response against a sublethal *C. pneumoniae* lung infection. Protective immunity is defined as an accelerated clearance of pulmonary infection.

Groups of 7 to 9 week old male Balb/c mice (8 to 10 per group) were immunized intramuscularly (i.m.) plus

30 intranasally (i.n.) with plasmid DNA containing each of the *C. pneumoniae* protein gene as described in Examples 1 and 2.



Saline or the plasmid vector lacking an inserted Chlamydial gene was given to groups of control animals.

For i.m. immunization, alternate left and right quadriceps were injected with 100µg of DNA in 50µl of PBS on three occasions at 0, 3 and 6 weeks. For i.n. immunization, anaesthetized mice were aspirated 50µl of PBS containing 50 µg DNA on three occasions at 0, 3 and 6 weeks. At week 8, immunized mice were inoculated i.n. with  $5 \times 10^5$  IFU of *C. pneumoniae*, strain AR39 in 100µl of SPG buffer to test their ability to limit the growth of a sublethal *C. pneumoniae* challenge.

Lungs were taken from mice at day 9 post-challenge and immediately homogenised in SPG buffer (7.5% sucrose, 5mM glutamate, 12.5mM phosphate pH7.5). The homogenate was stored frozen at -70°C until assay. Dilutions of the homogenate were assayed for the presence of infectious *Chlamydia* by inoculation onto monolayers of susceptible cells. The inoculum was centrifuged onto the cells at 3000rpm for 1 hour, then the cells were incubated for three days at 35°C in the presence of 1µg/ml cycloheximide. After incubation the monolayers were fixed with formalin and methanol then immunoperoxidase stained for the presence of Chlamydial inclusions using convalescent sera from rabbits infected with *C. pneumoniae* and metal-enhanced DAB as a peroxidase substrate.

#### 25 A. Immunization with pCACPNM213

Figure 31 and Table 1 show that mice immunized i.n. and i.m. with pCACPNM213 had chlamydial lung titers less than 60,000 in 3 of 6 cases at day 9 (mean 51,833) whereas the range of values for control mice sham immunized with saline was 34,200-377,800 IFU/lung (mean 141,450) at day 9. DNA immunisation per se was not responsible for the observed

protective effect since another plasmid DNA construct, pCACPNM102, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 153,283). The construct pCACPNM102 is identical to  
5 pCACPNM213 except that the nucleotide sequence encoding the putative ATP-binding cassette is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated ATP Synthase Subunit I protein.

#### B. Immunization with pCACPNM882

10 Figure 32 and Table 3 show that mice immunized i.n. and i.m. with pCACPNM882 had chlamydial lung titers less than 73,000 in 4 of 6 cases at day 9 (mean 77,500) whereas the range of values for control mice sham immunized with saline was 56,000-424,000 IFU/lung (mean 186,291) at day 9. DNA  
15 immunisation per se was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM647, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 143,883). The construct pCACPNM647 is identical to  
20 pCACPNM882 except that the nucleotide sequence encoding the putative Secretory locus ORF is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated substrate binding protein.

#### C. Immunization with pCACPNM208

25 Figure 33 and Table 4 show that mice immunized i.n. and i.m. with pCACPNM208 had chlamydial lung titers less than 67,000 in 4 of 6 cases at day 9 (mean 81,766) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 186,291) at day 9. DNA  
30 immunisation per se was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM647, failed to protect, with lung titers in immunised

mice similar to those obtained for saline-immunized control mice (mean 143,883). The construct pCACPNM647 is identical to pCACPNM208 except that the nucleotide sequence encoding the putative Endopeptidase is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated protein.

#### D. Immunization with pCACPNM1096

Figure 34 and Table 6 show that mice immunized i.n. and i.m. with pCACPNM1096 had chlamydial lung titers less than 30,000 in 5 of 6 cases at day 9 (mean 25,000) whereas the range of values for control mice sham immunized with saline was 51,300-170,000 IFU/lung (mean 105,150) at day 9. DNA immunisation *per se* was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM553, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 111,583). The construct pCACPNM553 is identical to pCACPNM1096 except that the nucleotide sequence encoding the putative Protease is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated protease.

#### E. Immunization with pCACPNM1097

Figure 35 and Table 8 show that mice immunized i.n. and i.m. with pCACPNM1097 had chlamydial lung titers less than 51,000 in 4 of 6 cases at day 9 (mean 62,883) whereas the range of values for control mice sham immunized with saline was 90,000-242,100 IFU/lung (mean 166,287) at day 9. DNA immunisation *per se* was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM1061, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 148,566). The construct pCACPNM1061 is identical to pCACPNM1097 except that the nucleotide sequence encoding the

putative Metalloprotease is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated zinc Metalloprotease.

#### F. Immunization with pCACPNM908

Figure 36 and Table 10 show that mice immunized i.n. and i.m. with pCACPNM908 had chlamydial lung titers less than 40,000 in 3 of 6 cases at day 9 (mean 68,333) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 207,962) at day 9. DNA immunisation per se was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM569, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 215,600). The construct pCACPNM569 is identical to pCACPNM908 except that the nucleotide sequence encoding the putative CLP protease ATPase is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated signal peptidase.

#### G. Immunization with pCACPNM909

Figure 37 and Table 12 show that mice immunized i.n. and i.m. with pCACPNM909 had chlamydial lung titers less than 85,000 in 5 of 6 cases at day 9 (mean 87,683) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 207,962) at day 9. DNA immunisation per se was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM569, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 215,600). The construct pCACPNM569 is identical to pCACPNM909 except that the nucleotide sequence encoding the putative CLP protease subunit is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated signal peptidase.

#### H. Immunization with pCACPNM440

Figure 38 and Table 14 show that mice immunized i.n. and i.m. with pCACPNM440 had chlamydial lung titers less than 98,000 in 4 of 6 cases at day 9 (mean 87,616) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 186,291) at day 9. DNA immunisation *per se* was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM647 failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 143,883). The construct pCACPNM647 is identical to pCACPNM440 except that the nucleotide sequence encoding the putative transglycolase /transpeptidase gene is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated gene.

#### I. Immunization with pCACPNM459

Figure 39 and Table 16 show that mice immunized i.n. and i.m. with pCACPNM459 had chlamydial lung titers less than 70,000 in 4 of 6 cases at day 9 (mean 70,516) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 186,291) at day 9. DNA immunisation *per se* was not responsible for the observed protective effect since another plasmid DNA construct, pCACPNM647, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 143,883). The construct pCACPNM647 is identical to pCACPNM459 except that the nucleotide sequence encoding the putative CLPc protease is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated gene.

#### J. Immunization with pCACPNM708

Figure 40 and Table 18 show that mice immunized i.n. and i.m. with pCACPNM708 had chlamydial lung titers less than

52,000 in 4 of 6 cases at day 9 (mean 73,916) whereas the range of values for control mice sham immunized with saline was 56,000-424,100 IFU/lung (mean 207,962) at day 9. DNA immunisation *per se* was not responsible for the observed  
5 protective effect since another plasmid DNA construct, pCACPNM569, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice (mean 215,600). The construct pCACPNM569 is identical to pCACPNM708 except that the nucleotide sequence encoding the  
10 putative thioredoxin is replaced with a *C. pneumoniae* nucleotide sequence encoding an unrelated *C. pneumoniae* gene.

Example 4:

This example illustrates the identification of B- and T-cell epitopes in proteins as expressed from each of  
15 pCACPNM213, pCACPNM882, pCACPNM208, pCACPNM1096, pCACPNM1097, pCACPNM909, pCACPNM440, pCACPNM459 and pCACPNM708.

B-cell epitopes were identified based on the product of flexibility and hydrophobicity propensities using the program SEQSEE (Wishart DS, et al. "SEQSEE: a comprehensive program  
20 suite for protein sequence analysis." *Comput Appl Biosci.* 1994 Apr;10(2):121-32) to identify external surface features (epitopes). T-cell epitopes for HLA-A0201 MHC subclass were identified based on the algorithm of Parker et al. 1995 (Parker KC, et al. "Peptide binding to MHC class I molecules:  
25 implications for antigenic peptide prediction." *Immunol Res* 1995;14(1):34-57). These epitopes are shown in Tables 2, 5, 7, 9, 11, 13, 15, 17 and 19 and SEQ ID NOs: 41 to 74.

Table 1

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPMM102	pCACPMM213
	Day 9	Day 9	Day 9
1	64900	207500	54200
2	116500	166500	10600
3	34200	114700	67400
4	377800	167400	32000
5	86200	179700	66900
6	206200	83900	79900
7	142600		
8	103200		
MEAN	141450	153283.333	51833.3333
SD	108598.7	45417.99	25908.35
Wilcoxon p		1.655	0.0293

Table 2: Identified B- T-cell epitopes from CPNM213

B cell epitope	T cell epitope
188 VHHTLRESYKKGTPPST (SEQ ID No: 41)	434 WIAEYVSPV (SEQ ID No: 43)
345 NLQKEISTEERQTKAR (SEQ ID No: 42)	

Table 3

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM647	pCACPNM882
	Day 9	Day 9	Day 9
1	209800	45100	18100
2	70000	222000	130300
3	226700	152500	72900
4	178900	89000	53500
5	424100	95500	63400
6	242200	259200	126800
7	256000		
8	56000		
9	173600		
10	185000		
11	121400		
12	91800		
MEAN	186291.667	143883.333	77500
SD	100263.3	83169.31	43686.75
Wilcoxon p		0.4936	0.0182



Table 4

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM647	pCACPNM208
	Day 9	Day 9	Day 9
1	209800	45100	142500
2	70000	222000	66900
3	226700	152500	58200
4	178900	89000	46500
5	424100	95500	110900
6	242200	259200	65600
7	256000		
8	56000		
9	173600		
10	185000		
11	121400		
12	91800		
MEAN	186291.667	143883.333	81766.6667
SD	100263.3	83169.31	36929.10
Wilcoxon p		0.4936	0.0135

Table 5 Identified B- T-cell epitopes from CPNM208

B cell epitope	T cell epitope
220 KGNSSPRSPAP (SEQ ID No: 44)	67 LLIEDMDLI (SEQ ID No: 46)
313 GENFQKNSS (SEQ ID No: 45)	66 NLLIEDMDL (SEQ ID No: 47)

Table 6

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM553	pCACPNM1096
	Day 9	Day 9	Day 9
1	136900	135600	21000
2	81700	112600	9700
3	119400	88600	28500
4	58500	121700	52000
5	110600	165300	17200
6	51300	45700	21600
7	170000		
8	112800		
MEAN	105150	111583.333	25000
SD	39876.3	41071.91	14585.88
Wilcoxon p		1.245	0.0013

Table 7 Identified B- T-cell epitopes from CPNM1096

B cell epitope	T cell epitope
328 TDLEGLEEDHKDSPWE (SEQ ID No: 48)	135 YLGDEILEV (SEQ ID No: 50)
589 SENAKKSEEQTSPQETPE (SEQ ID No: 49)	373 YLYSLLSML (SEQ ID No: 51)

Table 8

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM106 1	pCACPNM1097
	Day 9	Day 9	Day 9
1	232900	120800	50300
2	168100	184100	43900
3	105500	95600	65200
4	173100	147500	157900
5	90000	218700	22800
6	242100	124700	37200
7	183700		
8	134900		
MEAN	166287.5	148566.667	62883.3333
SD	54821.4	45450.00	48618.00
Wilcoxon p		0.662	0.0047

Table 9 Identified B- T-cell epitopes from CPNM1097

B cell epitope	T cell epitope
198 TTNRQKAL (SEQ ID No: 52)	207 SVLSRVNYV (SEQ ID No: 54)
221 VNSSNSNRLRE (SEQ ID No: 53)	279 KLSSLIPGL (SEQ ID No: 55)
	118 ILIGHKKHV (SEQ ID No: 56)

Table 10

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM569	pCACPNM908
	Day 9	Day 9	Day 9
1	209800	142800	37300
2	70000	420700	85000
3	226700	116600	35700
4	178900	161300	39700
5	424100	89200	123400
6	242200	363000	88900
7	256000		
8	56000		
MEAN	207962.5	215600	68333.3333
SD	115585.8	139870.70	36279.40
Wilcoxon p		0.8518	0.02

Table 11 Identified B- T-cell epitopes from CPNM908

B cell epitope	T cell epitope
226 PPKGGRKHPNQEYI (SEQ ID No: 57)	137 KILDVPFTI (SEQ ID No: 59)
273 SDDQADLSQKTRDH (SEQ ID No: 58)	168 LLQAADYDV (SEQ ID No: 60)

Table 12

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM569	PCACPNM909
	Day 9	Day 9	Day 9
1	209800	142800	206700
2	70000	420700	84700
3	226700	116600	81100
4	178900	161300	56700
5	424100	89200	53900
6	242200	363000	43000
7	256000		
8	56000		
MEAN	207962.5	215600	87683.3333
SD	115585.8	139870.70	60522.87
Wilcoxon p		0.8518	0.0426

Table 13 Identified B- T-cell epitopes from CPNM909

B cell epitope	T cell epitope
107 GTKGKRHAL (SEQ ID No: 61)	76 AIYDTIRFL (SEQ ID No: 63)
193 AKETNKDTSST (SEQ ID No: 62)	

**Table 14**

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPMM647	pCACPMM440
	Day 9	Day 9	Day 9
1	209800	45100	97200
2	70000	222000	92500
3	226700	152500	104400
4	178900	89000	60900
5	424100	95500	40400
6	242200	259200	130300
7	256000		
8	56000		
9	173600		
10	185000		
11	121400		
12	91800		
MEAN	186291.667	143883.333	87616.6667
SD	100263.3	83169.31	32132.31
Wilcoxon p		0.4936	0.0415

**Table 15 Identified B- T-cell epitopes from CPNM440**

B cell epitope	T cell epitope
287 DPTNYKEYFNNKERIEHTK (SEQ ID No: 64)	40 ALGQHEFCV (SEQ ID No: 66)
637 KRLYEENRSPKQGGTR (SEQ ID No: 65)	456 ILATGIQMV (SEQ ID No: 67)

Table 16

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPNM647	pCACPNM459
	Day 9	Day 9	Day 9
1	209800	45100	77400
2	70000	222000	60700
3	226700	152500	121000
4	178900	89000	68500
5	424100	95500	44800
6	242200	259200	50700
7	256000		
8	56000		
9	173600		
10	185000		
11	121400		
12	91800		
MEAN	186291.667	143883.333	70516.6667
SD	100263.3	83169.31	27387.69
Wilcoxon p		0.4936	0.0047

Table 17 Identified B- T-cell epitopes from CPNM459

B cell epitope	T cell epitope
467 DEEKKLRERLQSMKQEWENHKEEHQ (SEQ ID No: 68)	565 FLFLGPTGV (SEQ ID No: 70)
548 IRRSRTGIKDPNRPTG (SEQ ID No: 69)	410 FLPDKAIDL (SEQ ID No: 71)

Table 18

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS		
	IMMUNIZING CONSTRUCT		
	Saline	pCACPm569	pCACPm708
	Day 9	Day 9	Day 9
1	209800	142800	95100
2	70000	420700	189600
3	226700	116600	29000
4	178900	161300	51400
5	424100	89200	31500
6	242200	363000	46900
7	256000		
8	56000		
MEAN	207962.5	215600	73916.6667
SD	115585.8	139870.70	61457.22
Wilcoxon p		0.8518	0.0127

Table 19 Identified B- T-cell epitopes from pCPNM708

B cell epitope	T cell epitope
54 NIDENSKPAETYE (SEQ ID No: 72)	40 NLAAELPHV (SEQ ID No: 73)
	74 ILFKDGNV (SEQ ID No: 74)



CLAIMS:

1. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence set forth in any one of  
5 SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(iii) a nucleic acid sequence which encodes a  
10 polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in any one of SEQ ID  
15 Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid  
20 sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

2. A vaccine comprising a vaccine vector and at least  
25 one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20;

5 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

10 wherein each first nucleic acid is capable of being expressed.

3. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 1;

15 (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 1;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 1; and

20 (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 2;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains  
25 immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

4. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;

5 (ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide,  
10 wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

15 5. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

20 (i) SEQ ID No: 1;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 1;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
25 sequence to the polypeptide encoded by SEQ ID No: 1; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 2;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

6. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

7. The vaccine of claim 5 or 6 wherein the second polypeptide is a heterologous signal peptide.

8. The vaccine of claim 5 or 6 wherein the second polypeptide has adjuvant activity.
9. The vaccine of any one of claims 3 to 8 wherein wherein each first nucleic acid is operatively linked to one or  
5 more expression control sequences.
10. A vaccine according to any one of claims 3 to 9, further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.
- 10 11. The vaccine of claim 10 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.
12. A pharmaceutical composition comprising a vaccine according to any one of claims 3 to 11 and a pharmaceutically acceptable carrier.
- 15 13. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:
- (i) a polypeptide encoded by SEQ ID NO: 1;
  - (ii) a polypeptide which is at least 75% identical in  
20 amino acid sequence to SEQ ID NO: 2 or to the polypeptide encoded by SEQ ID NO: 1;
  - (iii) a polypeptide of SEQ ID NO: 2; and
  - (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is  
25 at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

14. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence  
5 comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;  
1;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;  
2;

10 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).  
15

15. The fusion protein of claim 13 or 14 wherein the  
15 second polypeptide is a heterologous signal peptide.

16. The fusion protein of claim 13 or 14 wherein the second polypeptide has adjuvant activity.

17. An antibody against the fusion protein of any one of claims 13 to 15.

20 18. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 1;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 2 or to the polypeptide  
25 encoded by SEQ ID NO: 1;

(iii) a polypeptide of SEQ ID NO: 2; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
5 (ii) or (iii).

19. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:  
10 1;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No:  
2;

(iii) a polypeptide as defined in (i) or (ii), which  
15 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

20. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second  
20 polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 1;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 2 or to the polypeptide  
25 encoded by SEQ ID NO: 1;

(iii) a polypeptide of SEQ ID NO: 2; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the

corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

21. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;

- 10 (ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

22. The vaccine of claim 20 or 21 wherein the second polypeptide is a heterologous signal peptide.

23. The vaccine of claim 20 or 21 wherein the second polypeptide has adjuvant activity.

24. A vaccine according to any one of claims 18 to 23, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

25. The vaccine according to claim 24 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

26. A pharmaceutical composition comprising a vaccine according to any one of claims 18 to 25 and a pharmaceutically acceptable carrier.



27. A pharmaceutical composition comprising an antibody according to claim 17 and a pharmaceutically acceptable carrier.

28. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 3 to 11 and 18 to 25.

29. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 12, 26 and 27.

30. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 13 to 16.

31. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the antibody of claim 17.

32. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 1;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 1;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 1; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 2;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified

polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

- 5 wherein each first nucleic acid is capable of being expressed; and instructions for use in eliciting an immunoprotective response in a mammal.

33. A commercial package comprising at least one nucleic acid selected from any one of:

- 10 (i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;

- 15 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

- 20 wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

34. A commercial package comprising at least one  
25 polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 1;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 2 or to the polypeptide encoded by SEQ ID NO: 1;

(iii) a polypeptide of SEQ ID NO: 2; and

5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii);

10 and instructions for use in eliciting an immunoprotective response in a mammal.

35. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence  
15 comprising at least 36 consecutive nucleotides from SEQ ID NO: 1;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 2;

20 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii);

and instructions for use in eliciting an immunoprotective  
25 response in a mammal.

36. Expression plasmid pCACPNM213 as shown in Figure 21.

37. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

(i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 41 to 43; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

38. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 41 to 43; and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

39. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 3;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 3;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 3; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 4;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

40. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 4;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

41. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 3;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 3;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
5 sequence to the polypeptide encoded by SEQ ID No: 3; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 4;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
10 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

15 wherein each first nucleic acid is capable of being expressed.

42. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid  
20 selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino  
25 acids from SEQ ID No: 4;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is

at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being  
5 expressed.

43. The vaccine of claim 41 or 42 wherein the second polypeptide is a heterologous signal peptide.

44. The vaccine of claim 41 or 42 wherein the second polypeptide has adjuvant activity.

10 45. The vaccine of any one of claims 39 to 44 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

46. A vaccine according to any one of claims 39 to 45, further comprising a second nucleic acid encoding an additional  
15 polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

47. The vaccine of claim 46 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

48. A pharmaceutical composition comprising a vaccine  
20 according to any one of claims 39 to 47 and a pharmaceutically acceptable carrier.

49. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

25 (i) a polypeptide encoded by SEQ ID NO: 3;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 4 or to the polypeptide encoded by SEQ ID NO: 3;

(iii) a polypeptide of SEQ ID NO: 4; and

5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

10 50. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:  
15 3;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No:  
4;

(iii) a polypeptide as defined in (i) or (ii), which  
20 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

51. The fusion protein of claim 49 or 50 wherein the second polypeptide is a heterologous signal peptide.

25 52. The fusion protein of claim 49 or 50 wherein the second polypeptide has adjuvant activity.

53. An antibody against the fusion protein of any one of claims 49 to 51.



54. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 3;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 4 or to the polypeptide encoded by SEQ ID NO: 3;

(iii) a polypeptide of SEQ ID NO: 4; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

55. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 4;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

56. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 3;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 4 or to the polypeptide encoded by SEQ ID NO: 3;

5 (iii) a polypeptide of SEQ ID NO: 4; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),

10 (ii) or (iii).

57. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

15 (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 4;

20 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

25 58. The vaccine of claim 56 or 57 wherein the second polypeptide is a heterologous signal peptide.

59. The vaccine of claim 56 or 57 wherein the second polypeptide has adjuvant activity.

60. A vaccine according to any one of claims 54 or 59, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

61. The vaccine according to claim 60 wherein the  
5 additional polypeptide comprises a *Chlamydia* polypeptide.

62. A pharmaceutical composition comprising a vaccine according to any one of claims 54 to 61 and a pharmaceutically acceptable carrier.

63. A pharmaceutical composition comprising an antibody  
10 according to claim 53 and a pharmaceutically acceptable carrier.

64. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 39 to 47 and 54 to  
15 61.

65. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 48, 62 and 63.

66. A method for preventing or treating *Chlamydia*  
20 infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 49 to 52.

67. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the antibody of claim 53.

25 68. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 3;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 3;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
5 sequence to the polypeptide encoded by SEQ ID No: 3; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 4;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
10 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being  
15 expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

69. A commercial package comprising at least one nucleic acid selected from any one of:

20 (i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 4;

25 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

- 5 70. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 3;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 4 or to the polypeptide  
10 encoded by SEQ ID NO: 3;

(iii) a polypeptide of SEQ ID NO: 4; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
15 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

71. A commercial package comprising at least one  
20 polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 3;

(ii) a polypeptide which is an immunogenic fragment  
25 comprising at least 12 consecutive amino acids from SEQ ID No: 4;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii);

- 5 and instructions for use in eliciting an immunoprotective response in a mammal.

72. Expression plasmid pCACPNM882 as shown in Figure 22.

73. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

- 10 (i) SEQ ID No: 5;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 5;

- (iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
15 sequence to the polypeptide encoded by SEQ ID No: 5; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 6;

- (v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
20 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

- wherein each first nucleic acid is capable of being  
25 expressed.

74. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 5;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 6;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

75. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 5;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 5;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 5; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 6;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains

immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

5 wherein each first nucleic acid is capable of being expressed.

76. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid  
10 selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 5;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino  
15 acids from SEQ ID No: 6;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the  
20 corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

77. The vaccine of claim 75 or 76 wherein the second  
25 polypeptide is a heterologous signal peptide.

78. The vaccine of claim 75 or 76 wherein the second polypeptide has adjuvant activity.



79. The vaccine of any one of claims 73 to 78 wherein each first nucleic acid is operatively linked to one or more expression control sequences.
80. A vaccine according to any one of claims 73 to 79, further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.
81. The vaccine of claim 80 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.
- 10 82. A pharmaceutical composition comprising a vaccine according to any one of claims 73 to 81 and a pharmaceutically acceptable carrier.
83. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any  
15 one of:
- (i) a polypeptide encoded by SEQ ID NO: 5;
  - (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 6 or to the polypeptide encoded by SEQ ID NO: 5;
  - 20 (iii) a polypeptide of SEQ ID NO: 6; and
  - (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
25 (ii) or (iii).
84. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 5;

(ii) a polypeptide which is an immunogenic fragment  
5 comprising at least 12 consecutive amino acids from SEQ ID No: 6;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding  
10 polypeptide of (i) or (ii).

85. The fusion protein of claim 83 or 84 wherein the second polypeptide is a heterologous signal peptide.

86. The fusion protein of claim 83 or 84 wherein the second polypeptide has adjuvant activity.

15 87. An antibody against the fusion protein of any one of claims 83 to 85.

88. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 5;

20 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 6 or to the polypeptide encoded by SEQ ID NO: 5;

(iii) a polypeptide of SEQ ID NO: 6; and

(iv) a polypeptide as defined in (i), (ii) or (iii)  
25 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

89. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 5;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 6;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

90. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second  
15 polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 5;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 6 or to the polypeptide  
20 encoded by SEQ ID NO: 5;

(iii) a polypeptide of SEQ ID NO: 6; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
25 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

91. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second

polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 5;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 6;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

92. The vaccine of claim 90 or 91 wherein the second polypeptide is a heterologous signal peptide.

15 93. The vaccine of claim 90 or 91 wherein the second polypeptide has adjuvant activity.

94. A vaccine according to any one of claims 88 to 93, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

20 95. The vaccine according to claim 94 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

96. A pharmaceutical composition comprising a vaccine according to any one of claims 88 to 95 and a pharmaceutically acceptable carrier.

25 97. A pharmaceutical composition comprising an antibody according to claim 87 and a pharmaceutically acceptable carrier.

98. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 73 to 81 and 88 to 95.
- 5 99. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 82, 96 and 97.
100. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective  
10 amount of the fusion protein of any one of claims 83 to 86.
101. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the antibody of claim 87.
102. A commercial package comprising at least one nucleic  
15 acid selected from any one of:
- (i) SEQ ID No: 5;
  - (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 5;
  - (iii) a nucleic acid sequence which encodes a  
20 polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 5; and
  - (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 6;
  - (v) a nucleic acid sequence as defined in (i), (ii)  
25 or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

- 5 103. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 5;

- (ii) a nucleic acid sequence which encodes an  
10 immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 6;

- (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is  
15 at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

- instructions for use in eliciting an immunoprotective  
20 response in a mammal.

104. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 5;

- (ii) a polypeptide which is at least 75% identical in  
25 amino acid sequence to SEQ ID NO: 6 or to the polypeptide encoded by SEQ ID NO: 5;

(iii) a polypeptide of SEQ ID NO: 6; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
5 (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

105. A commercial package comprising at least one polypeptide selected from any one of:

10 (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 5;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No:  
15 6;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

20 instructions for use in eliciting an immunoprotective response in a mammal.

106. Expression plasmid pCACPNM208 as shown in Figure 23.

107. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

25 (i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 44 to 47; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively

substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

- 5 108. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 44 to 47;

and

- (ii) a polypeptide as defined in (i) which has been  
10 modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

109. A vaccine comprising a vaccine vector and at least  
15 one first nucleic acid selected from any one of:

(i) SEQ ID No: 7;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 7;

- (iii) a nucleic acid sequence which encodes a  
20 polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 7; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 8;

- (v) a nucleic acid sequence as defined in (i), (ii)  
25 or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);



wherein each first nucleic acid is capable of being expressed.

110. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

5 (i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

10 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

15 wherein each first nucleic acid is capable of being expressed.

111. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

20 (a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 7;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 7;

25 (iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 7; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 8;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

112. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

113. The vaccine of claim 111 or 112 wherein the second polypeptide is a heterologous signal peptide.

5 114. The vaccine of claim 111 or 112 wherein the second polypeptide has adjuvant activity.

115. The vaccine of any one of claims 109 to 114 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

10 116. A vaccine according to any one of claims 109 to 115, further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

117. The vaccine of claim 116 wherein the second nucleic  
15 acid encodes an additional *Chlamydia* polypeptide.

118. A pharmaceutical composition comprising a vaccine according to any one of claims 109 to 117 and a pharmaceutically acceptable carrier.

119. A fusion protein comprising a first and a second  
20 polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 7;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 8 or to the polypeptide  
25 encoded by SEQ ID NO: 7;

(iii) a polypeptide of SEQ ID NO: 8; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
5 (ii) or (iii).

120. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence  
10 comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

15 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

121. The fusion protein of claim 119 or 120 wherein the  
20 second polypeptide is a heterologous signal peptide.

122. The fusion protein of claim 119 or 120 wherein the second polypeptide has adjuvant activity.

123. An antibody against the fusion protein of any one of claims 119 to 121.

25 124. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 7;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 8 or to the polypeptide encoded by SEQ ID NO: 7;

(iii) a polypeptide of SEQ ID NO: 8; and

- 5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

- 10 125. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

- 15 (ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

- (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at  
20 least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

126. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any  
25 one of:

(i) a polypeptide encoded by SEQ ID NO: 7;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 8 or to the polypeptide encoded by SEQ ID NO: 7;

(iii) a polypeptide of SEQ ID NO: 8; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
5 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

127. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any  
10 one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

(ii) a polypeptide which is an immunogenic fragment  
15 comprising at least 12 consecutive amino acids from SEQ ID No: 8;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding  
20 polypeptide of (i) or (ii).

128. The vaccine of claim 126 or 127 wherein the second polypeptide is a heterologous signal peptide.

129. The vaccine of claim 126 or 127 wherein the second polypeptide has adjuvant activity.

25 130. A vaccine according to any one of claims 124 to 129, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

131. The vaccine according to claim 130 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

132. A pharmaceutical composition comprising a vaccine according to any one of claims 124 to 131 and a pharmaceutically acceptable carrier.

133. A pharmaceutical composition comprising an antibody  
5 according to claim 123 and a pharmaceutically acceptable carrier.

134. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 109 to 117 and 124  
10 to 131.

135. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 118, 132 and 133.

15 136. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 119 to 122.

137. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective  
20 amount of the antibody of claim 123.

138. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 7;

(ii) a nucleic acid sequence which encodes a  
25 polypeptide encoded by SEQ ID No: 7;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 7; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 8;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

139. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.



140. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 7;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 8 or to the polypeptide encoded by SEQ ID NO: 7;

(iii) a polypeptide of SEQ ID NO: 8; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

15 141. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 7;

20 (ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 8;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii);

25 and instructions for use in eliciting an immunoprotective response in a mammal.

142. Expression plasmid pCACPNI096 as shown in Figure 24.

143. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

(i) a nucleic acid encoding a polypeptide of any one  
5 of SEQ ID Nos: 48 to 51; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino  
10 acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

144. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 48 to 51;  
15 and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding  
20 polypeptide encoded by the nucleic acid of (i).

145. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 9;

(ii) a nucleic acid sequence which encodes a  
25 polypeptide encoded by SEQ ID No: 9;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 9; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 10;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

146. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 10;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

147. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 9;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 9;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 9; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 10;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

148. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 10;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the  
5 corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

149. The vaccine of claim 147 or 148 wherein the second  
10 polypeptide is a heterologous signal peptide.

150. The vaccine of claim 147 or 148 wherein the second polypeptide has adjuvant activity.

151. The vaccine of any one of claims 145 to 150 wherein each first nucleic acid is operatively linked to one or more  
15 expression control sequences.

152. A vaccine according to any one of claims 145 to 151, further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

20 153. The vaccine of claim 152 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

154. A pharmaceutical composition comprising a vaccine according to any one of claims 145 to 153 and a pharmaceutically acceptable carrier.

25 155. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 9;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 10 or to the polypeptide encoded by SEQ ID NO: 9;

(iii) a polypeptide of SEQ ID NO: 10; and

- 5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

- 10 156. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

- 15 9;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 10;

- 20 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

157. The fusion protein of claim 155 or 156 wherein the second polypeptide is a heterologous signal peptide.

- 25 158. The fusion protein of claim 155 or 156 wherein the second polypeptide has adjuvant activity.

159. An antibody against the fusion protein of any one of claims 155 to 157.

160. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 9;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 10 or to the polypeptide encoded by SEQ ID NO: 9;

(iii) a polypeptide of SEQ ID NO: 10; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

161. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 10;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

162. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 9;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 10 or to the polypeptide encoded by SEQ ID NO: 9;

5 (iii) a polypeptide of SEQ ID NO: 10; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
10 (ii) or (iii).

163. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

15 (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No:  
20 10;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

25 164. The vaccine of claim 162 or 163 wherein the second polypeptide is a heterologous signal peptide.

165. The vaccine of claim 162 or 163 wherein the second polypeptide has adjuvant activity.



166. A vaccine according to any one of claims 160 to 165, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

167. The vaccine according to claim 166 wherein the  
5 additional polypeptide comprises a *Chlamydia* polypeptide.

168. A pharmaceutical composition comprising a vaccine according to any one of claims 160 to 167 and a pharmaceutically acceptable carrier.

169. A pharmaceutical composition comprising an antibody  
10 according to claim 159 and a pharmaceutically acceptable carrier.

170. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 145 to 153 and 160  
15 to 167.

171. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 154, 168 and 169.

20 172. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 155 to 158.

173. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective  
25 amount of the antibody of claim 159.

174. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 9;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 9;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
5 sequence to the polypeptide encoded by SEQ ID No: 9; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 10;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
10 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being  
15 expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

175. A commercial package comprising at least one nucleic acid selected from any one of:

20 (i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 10;

25 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

- 5 176. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 9;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 10 or to the polypeptide  
10 encoded by SEQ ID NO: 9;

(iii) a polypeptide of SEQ ID NO: 10; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
15 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

177. A commercial package comprising at least one  
20 polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 9;

(ii) a polypeptide which is an immunogenic fragment  
25 comprising at least 12 consecutive amino acids from SEQ ID No: 10;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

5 instructions for use in eliciting an immunoprotective response in a mammal.

178. Expression plasmid pCACPNM1097 as shown in Figure 25.

179. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

10 (i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 52 to 56; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide  
15 retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

180. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

20 (i) a polypeptide of any one of SEQ ID Nos: 52 to 56; and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75%  
25 identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

181. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 11;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 11;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 11; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 12;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

182. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 11;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

183. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the  
5 fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 11;

(ii) a nucleic acid sequence which encodes a  
10 polypeptide encoded by SEQ ID No: 11;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 11; and

(iv) a nucleic acid sequence which encodes a  
15 polypeptide whose sequence is set forth in SEQ ID No: 12;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid  
20 sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

184. A vaccine comprising a vaccine vector and at least  
25 one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 11;

5 (ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide,  
10 wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

15 wherein each first nucleic acid is capable of being expressed.

185. The vaccine of claim 183 or 184 wherein the second polypeptide is a heterologous signal peptide.

186. The vaccine of claim 183 or 184 wherein the second polypeptide has adjuvant activity.

20 187. The vaccine of any one of claims 181 to 186 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

188. A vaccine according to any one of claims 181 to 187, further comprising a second nucleic acid encoding an additional  
25 polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

189. The vaccine of claim 188 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

190. A pharmaceutical composition comprising a vaccine according to any one of claims 181 to 189 and a pharmaceutically acceptable carrier.

191. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 11;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 12 or to the polypeptide encoded by SEQ ID NO: 11;

(iii) a polypeptide of SEQ ID NO: 12; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

192. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 11;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).



193. The fusion protein of claim 191 or 192 wherein the second polypeptide is a heterologous signal peptide.

194. The fusion protein of claim 191 or 192 wherein the second polypeptide has adjuvant activity.

5 195. An antibody against the fusion protein of any one of claims 191 to 193.

196. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 11;

10 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 12 or to the polypeptide encoded by SEQ ID NO: 11;

(iii) a polypeptide of SEQ ID NO: 12; and

(iv) a polypeptide as defined in (i), (ii) or (iii)  
15 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

197. A vaccine comprising at least one first polypeptide  
20 selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 11;

(ii) a polypeptide which is an immunogenic fragment  
25 comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

- 5 198. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 11;

- 10 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 12 or to the polypeptide encoded by SEQ ID NO: 11;

(iii) a polypeptide of SEQ ID NO: 12; and

- (iv) a polypeptide as defined in (i), (ii) or (iii)  
15 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

199. A vaccine comprising at least one fusion protein,  
20 wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

- (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:  
25 11;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

5 200. The vaccine of claim 198 or 199 wherein the second polypeptide is a heterologous signal peptide.

201. The vaccine of claim 198 or 199 wherein the second polypeptide has adjuvant activity.

202. A vaccine according to any one of claims 196 to 201,  
10 further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

203. The vaccine according to claim 202 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

204. A pharmaceutical composition comprising a vaccine  
15 according to any one of claims 196 to 203 and a pharmaceutically acceptable carrier.

205. A pharmaceutical composition comprising an antibody according to claim 195 and a pharmaceutically acceptable carrier.

20 206. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 181 to 189 and 196 to 203.

207. A method for preventing or treating *Chlamydia*  
25 infection comprising administering to a mammal an effective amount of the composition of any one of claims 190, 204 and 205.

208. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 191 to 194.

209. A method for preventing or treating *Chlamydia*  
5 infection comprising administering to a mammal an effective amount of the antibody of claim 195.

210. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 11;

10 (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 11;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 11; and

15 (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 12;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains  
20 immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

25 instructions for use in eliciting an immunoprotective response in a mammal.

211. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 11;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

212. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 11;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 12 or to the polypeptide encoded by SEQ ID NO: 11;

(iii) a polypeptide of SEQ ID NO: 12; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

213. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 11;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 12;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

instructions for use in eliciting an immunoprotective response in a mammal.

15 214. Expression plasmid pCACPNM908 as shown in Figure 26.

215. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

(i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 57 to 60; and

20 (ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the  
25 nucleic acid of (i).

216. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 57 to 60;  
and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified  
5 polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

217. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

10 (i) SEQ ID No: 13;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 13;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
15 sequence to the polypeptide encoded by SEQ ID No: 13; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 14;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
20 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being  
25 expressed.

218. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 13;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 14;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

219. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 13;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 13;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 13; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 14;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains



immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

5 wherein each first nucleic acid is capable of being expressed.

220. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid  
10 selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 13;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino  
15 acids from SEQ ID No: 14;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the  
20 corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

221. The vaccine of claim 219 or 220 wherein the second  
25 polypeptide is a heterologous signal peptide.

222. The vaccine of claim 219 or 220 wherein the second polypeptide has adjuvant activity.

223. The vaccine of any one of claims 217 to 222 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

224. A vaccine according to any one of claims 217 to 223,  
5 further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

225. The vaccine of claim 224 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

10 226. A pharmaceutical composition comprising a vaccine according to any one of claims 217 to 225 and a pharmaceutically acceptable carrier.

227. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any  
15 one of:

(i) a polypeptide encoded by SEQ ID NO: 13;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 14 or to the polypeptide encoded by SEQ ID NO: 13;

20 (iii) a polypeptide of SEQ ID NO: 14; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
25 (ii) or (iii).

228. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 13;

(ii) a polypeptide which is an immunogenic fragment  
5 comprising at least 12 consecutive amino acids from SEQ ID No: 14;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding  
10 polypeptide of (i) or (ii).

229. The fusion protein of claim 227 or 228 wherein the second polypeptide is a heterologous signal peptide.

230. The fusion protein of claim 227 or 228 wherein the second polypeptide has adjuvant activity.

15 231. An antibody against the fusion protein of any one of claims 227 to 229.

232. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 13;

20 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 14 or to the polypeptide encoded by SEQ ID NO: 13;

(iii) a polypeptide of SEQ ID NO: 14; and

(iv) a polypeptide as defined in (i), (ii) or (iii)  
25 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

233. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 13;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 14;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

234. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second  
15 polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 13;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 14 or to the polypeptide  
20 encoded by SEQ ID NO: 13;

(iii) a polypeptide of SEQ ID NO: 14; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
25 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

235. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second

polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 13;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 14;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

236. The vaccine of claim 234 or 235 wherein the second polypeptide is a heterologous signal peptide.

15 237. The vaccine of claim 234 or 235 wherein the second polypeptide has adjuvant activity.

238. A vaccine according to any one of claims 232 to 237, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

20 239. The vaccine according to claim 238 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

240. A pharmaceutical composition comprising a vaccine according to any one of claims 232 to 239 and a pharmaceutically acceptable carrier.

25 241. A pharmaceutical composition comprising an antibody according to claim 231 and a pharmaceutically acceptable carrier.

242. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 217 to 225 and 232 to 239.
- 5 243. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 226, 240 and 241.
244. A method for preventing or treating *Chlamydia*  
10 infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 227 to 230.
245. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the antibody of claim 231.
- 15 246. A commercial package comprising at least one nucleic acid selected from any one of:
- (i) SEQ ID No: 13;
  - (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 13;
  - 20 (iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 13; and
  - (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 14;
  - 25 (v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid

sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

5 instructions for use in eliciting an immunoprotective response in a mammal.

247. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36  
10 consecutive nucleotides from SEQ ID NO: 13;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 14;

(iii) a nucleic acid sequence as defined in (i) or  
15 (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being  
20 expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

248. A commercial package comprising at least one polypeptide selected from any one of:

25 (i) a polypeptide encoded by SEQ ID NO: 13;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 14 or to the polypeptide encoded by SEQ ID NO: 13;

(iii) a polypeptide of SEQ ID NO: 14; and

5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

10 instructions for use in eliciting an immunoprotective response in a mammal.

249. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence  
15 comprising at least 36 consecutive nucleotides from SEQ ID NO: 13;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 14;

20 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

25 instructions for use in eliciting an immunoprotective response in a mammal.

250. Expression plasmid pCACPNM909 as shown in Figure 27.

251. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:



(i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 61 to 63; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

252. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 61 to 63; and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

253. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 15;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 15;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 15; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 16;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

254. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 16;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

255. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 15;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 15;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
5 sequence to the polypeptide encoded by SEQ ID No: 15; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 16;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
10 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

15 wherein each first nucleic acid is capable of being expressed.

256. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

20 (a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;

(ii) a nucleic acid sequence which encodes an  
25 immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 16;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide,

wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

5            wherein each first nucleic acid is capable of being expressed.

257.        The vaccine of claim 255 or 256 wherein the second polypeptide is a heterologous signal peptide.

258.        The vaccine of claim 255 or 256 wherein the second  
10           polypeptide has adjuvant activity.

259.        The vaccine of any one of claims 253 to 258 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

260.        A vaccine according to any one of claims 253 to 259,  
15           further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

261.        The vaccine of claim 260 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

20           262.        A pharmaceutical composition comprising a vaccine according to any one of claims 253 to 261 and a pharmaceutically acceptable carrier.

263.        A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any  
25           one of:

(i) a polypeptide encoded by SEQ ID NO: 15;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 16 or to the polypeptide encoded by SEQ ID NO: 15;

(iii) a polypeptide of SEQ ID NO: 16; and

5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

10 264. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

15 15;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 16;

(iii) a polypeptide as defined in (i) or (ii), which  
20 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

265. The fusion protein of claim 263 or 264 wherein the second polypeptide is a heterologous signal peptide.

25 266. The fusion protein of claim 263 or 264 wherein the second polypeptide has adjuvant activity.

267. An antibody against the fusion protein of any one of claims 263 to 265.

268. A vaccine comprising at least one first polypeptide selected from any one of:

- (i) a polypeptide encoded by SEQ ID NO: 15;
- (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 16 or to the polypeptide encoded by SEQ ID NO: 15;
- (iii) a polypeptide of SEQ ID NO: 16; and
- (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

269. A vaccine comprising at least one first polypeptide selected from any one of:

- (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;
- (ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 16;
- (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

270. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

- (i) a polypeptide encoded by SEQ ID NO: 15;
- (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 16 or to the polypeptide encoded by SEQ ID NO: 15;
- 5 (iii) a polypeptide of SEQ ID NO: 16; and
- (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),
- 10 (ii) or (iii).
271. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:
- 15 (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;
- (ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No:
- 20 16;
- (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).
- 25 272. The vaccine of claim 270 or 271 wherein the second polypeptide is a heterologous signal peptide.
273. The vaccine of claim 270 or 271 wherein the second polypeptide has adjuvant activity.

274. A vaccine according to any one of claims 268 to 273, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

275. The vaccine according to claim 274 wherein the  
5 additional polypeptide comprises a *Chlamydia* polypeptide.

276. A pharmaceutical composition comprising a vaccine according to any one of claims 268 to 275 and a pharmaceutically acceptable carrier.

277. A pharmaceutical composition comprising an antibody  
10 according to claim 267 and a pharmaceutically acceptable carrier.

278. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 253 to 261 and 268  
15 to 275.

279. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 262, 276 and 277.

20 280. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 263 to 266.

281. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective  
25 amount of the antibody of claim 267.

282. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 15;



(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 15;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid  
5 sequence to the polypeptide encoded by SEQ ID No: 15; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 16;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified  
10 polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being  
15 expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

283. A commercial package comprising at least one nucleic acid selected from any one of:

20 (i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 16;

25 (iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

- 5 284. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 15;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 16 or to the polypeptide  
10 encoded by SEQ ID NO: 15;

(iii) a polypeptide of SEQ ID NO: 16; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
15 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

instructions for use in eliciting an immunoprotective response in a mammal.

285. A commercial package comprising at least one  
20 polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 15;

(ii) a polypeptide which is an immunogenic fragment  
25 comprising at least 12 consecutive amino acids from SEQ ID No: 16;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

5 instructions for use in eliciting an immunoprotective response in a mammal.

286. Expression plasmid pCACPNM440 as shown in Figure 28.

287. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

10 (i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 64 to 67; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide  
15 retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

288. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

20 (i) a polypeptide of any one of SEQ ID Nos: 64 to 67; and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75%  
25 identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

289. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) SEQ ID No: 17;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 17;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 17; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 18;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

290. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 17;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

291. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the  
5 fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 17;

(ii) a nucleic acid sequence which encodes a  
10 polypeptide encoded by SEQ ID No: 17;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 17; and

(iv) a nucleic acid sequence which encodes a  
15 polypeptide whose sequence is set forth in SEQ ID No: 18;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid  
20 sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

292. A vaccine comprising a vaccine vector and at least  
25 one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 17;

5 (ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, 10 wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

15 wherein each first nucleic acid is capable of being expressed.

293. The vaccine of claim 291 or 292 wherein the second polypeptide is a heterologous signal peptide.

294. The vaccine of claim 291 or 292 wherein the second polypeptide has adjuvant activity.

20 295. The vaccine of any one of claims 289 to 294 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

296. A vaccine according to any one of claims 289 to 295, further comprising a second nucleic acid encoding an additional 25 polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

297. The vaccine of claim 296 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

298. A pharmaceutical composition comprising a vaccine according to any one of claims 289 to 297 and a pharmaceutically acceptable carrier.

299. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 17;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 18 or to the polypeptide encoded by SEQ ID NO: 17;

(iii) a polypeptide of SEQ ID NO: 18; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

300. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 17;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

301. The fusion protein of claim 299 or 300 wherein the second polypeptide is a heterologous signal peptide.

302. The fusion protein of claim 299 or 300 wherein the second polypeptide has adjuvant activity.

5 303. An antibody against the fusion protein of any one of claims 299 to 301.

304. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 17;

10 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 18 or to the polypeptide encoded by SEQ ID NO: 17;

(iii) a polypeptide of SEQ ID NO: 18; and

(iv) a polypeptide as defined in (i), (ii) or (iii)  
15 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

305. A vaccine comprising at least one first polypeptide  
20 selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 17;

(ii) a polypeptide which is an immunogenic fragment  
25 comprising at least 12 consecutive amino acids from SEQ ID No: 18;



(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

- 5 306. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 17;

- 10 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 18 or to the polypeptide encoded by SEQ ID NO: 17;

(iii) a polypeptide of SEQ ID NO: 18; and

- (iv) a polypeptide as defined in (i), (ii) or (iii)  
15 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

307. A vaccine comprising at least one fusion protein,  
20 wherein the fusion protein comprises a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:  
25 17;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

5 308. The vaccine of claim 306 or 307 wherein the second polypeptide is a heterologous signal peptide.

309. The vaccine of claim 306 or 307 wherein the second polypeptide has adjuvant activity.

310. A vaccine according to any one of claims 304 to 309,  
10 further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

311. The vaccine according to claim 310 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

312. A pharmaceutical composition comprising a vaccine  
15 according to any one of claims 304 to 311 and a pharmaceutically acceptable carrier.

313. A pharmaceutical composition comprising an antibody according to claim 303 and a pharmaceutically acceptable carrier.

20 314. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 289 to 297 and 304 to 311.

315. A method for preventing or treating *Chlamydia*  
25 infection comprising administering to a mammal an effective amount of the composition of any one of claims 298, 312 and 313.

316. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 299 to 302.

317. A method for preventing or treating *Chlamydia*  
5 infection comprising administering to a mammal an effective amount of the antibody of claim 303.

318. A commercial package comprising at least one nucleic acid selected from any one of:

(i) SEQ ID No: 17;

10 (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 17;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 17; and

15 (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 18;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains  
20 immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

25 instructions for use in eliciting an immunoprotective response in a mammal.

319. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 17;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

320. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 17;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 18 or to the polypeptide encoded by SEQ ID NO: 17;

(iii) a polypeptide of SEQ ID NO: 18; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii);

and instructions for use in eliciting an immunoprotective response in a mammal.

321. A commercial package comprising at least one polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 17;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 18;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii);

and instructions for use in eliciting an immunoprotective response in a mammal.

15 322. Expression plasmid pCACPNM459 as shown in Figure 29.

323. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

(i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 68 to 71; and

20 (ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the  
25 nucleic acid of (i).

324. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 68 to 71;  
and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

325. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

10 (i) SEQ ID No: 19;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 19;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 19; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 20;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed.

326. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 19;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 20;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being expressed.

327. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid selected from any one of:

(i) SEQ ID No: 19;

(ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 19;

(iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 19; and

(iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 20;

(v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains

immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv); and

(b) a second polypeptide;

5 wherein each first nucleic acid is capable of being expressed.

328. A vaccine comprising a vaccine vector and at least one first nucleic acid encoding a fusion protein, wherein the fusion protein comprises:

(a) a first polypeptide encoded by a nucleic acid  
10 selected from any one of:

(i) a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 19;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino  
15 acids from SEQ ID No: 20;

(iii) a nucleic acid sequence as defined in (i) or (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the  
20 corresponding fragment of (i) or (ii); and

(b) a second polypeptide;

wherein each first nucleic acid is capable of being expressed.

329. The vaccine of claim 327 or 328 wherein the second  
25 polypeptide is a heterologous signal peptide.

330. The vaccine of claim 327 or 328 wherein the second polypeptide has adjuvant activity.



331. The vaccine of any one of claims 325 to 330 wherein wherein each first nucleic acid is operatively linked to one or more expression control sequences.

332. A vaccine according to any one of claims 325 to 331,  
5 further comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by the first nucleic acid.

333. The vaccine of claim 332 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

10 334. A pharmaceutical composition comprising a vaccine according to any one of claims 325 to 333 and a pharmaceutically acceptable carrier.

335. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any  
15 one of:

(i) a polypeptide encoded by SEQ ID NO: 19;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 20 or to the polypeptide encoded by SEQ ID NO: 19;

20 (iii) a polypeptide of SEQ ID NO: 20; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i),  
25 (ii) or (iii).

336. A fusion protein comprising a first and a second polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 19;

(ii) a polypeptide which is an immunogenic fragment  
5 comprising at least 12 consecutive amino acids from SEQ ID No: 20;

(iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding  
10 polypeptide of (i) or (ii).

337. The fusion protein of claim 335 or 336 wherein the second polypeptide is a heterologous signal peptide.

338. The fusion protein of claim 335 or 336 wherein the second polypeptide has adjuvant activity.

15 339. An antibody against the fusion protein of any one of claims 335 to 337.

340. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 19;

20 (ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 20 or to the polypeptide encoded by SEQ ID NO: 19;

(iii) a polypeptide of SEQ ID NO: 20; and

(iv) a polypeptide as defined in (i), (ii) or (iii)  
25 which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

341. A vaccine comprising at least one first polypeptide selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 19;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 20;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

342. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second  
15 polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by SEQ ID NO: 19;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 20 or to the polypeptide  
20 encoded by SEQ ID NO: 19;

(iii) a polypeptide of SEQ ID NO: 20; and

(iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the  
25 corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii).

343. A vaccine comprising at least one fusion protein, wherein the fusion protein comprises a first and a second

polypeptide, wherein the first polypeptide is selected from any one of:

(i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO:

5 19;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 20;

(iii) a polypeptide as defined in (i) or (ii), which  
10 has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii).

344. The vaccine of claim 342 or 343 wherein the second polypeptide is a heterologous signal peptide.

15 345. The vaccine of claim 342 or 343 wherein the second polypeptide has adjuvant activity.

346. A vaccine according to any one of claims 340 to 345, further comprising an additional polypeptide which enhances the immune response to the first polypeptide.

20 347. The vaccine according to claim 346 wherein the additional polypeptide comprises a *Chlamydia* polypeptide.

348. A pharmaceutical composition comprising a vaccine according to any one of claims 340 to 347 and a pharmaceutically acceptable carrier.

25 349. A pharmaceutical composition comprising an antibody according to claim 339 and a pharmaceutically acceptable carrier.

350. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the vaccine of any one of claims 325 to 333 and 340 to 347.
- 5 351. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the composition of any one of claims 334, 348 and 349.
352. A method for preventing or treating *Chlamydia*  
10 infection comprising administering to a mammal an effective amount of the fusion protein of any one of claims 335 to 338.
353. A method for preventing or treating *Chlamydia* infection comprising administering to a mammal an effective amount of the antibody of claim 339.
- 15 354. A commercial package comprising at least one nucleic acid selected from any one of:
- (i) SEQ ID No: 19;
  - (ii) a nucleic acid sequence which encodes a polypeptide encoded by SEQ ID No: 19;
  - 20 (iii) a nucleic acid sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptide encoded by SEQ ID No: 19; and
  - (iv) a nucleic acid sequence which encodes a polypeptide whose sequence is set forth in SEQ ID No: 20;
  - 25 (v) a nucleic acid sequence as defined in (i), (ii) or (iv), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid

sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iv);

wherein each first nucleic acid is capable of being expressed; and

5 instructions for use in eliciting an immunoprotective response in a mammal.

355. A commercial package comprising at least one nucleic acid selected from any one of:

(i) a nucleic acid sequence comprising at least 36  
10 consecutive nucleotides from SEQ ID NO: 19;

(ii) a nucleic acid sequence which encodes an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 20;

(iii) a nucleic acid sequence as defined in (i) or  
15 (ii), which has been modified to encode a modified polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding fragment of (i) or (ii);

wherein each first nucleic acid is capable of being  
20 expressed; and

instructions for use in eliciting an immunoprotective response in a mammal.

356. A commercial package comprising at least one polypeptide selected from any one of:

25 (i) a polypeptide encoded by SEQ ID NO: 19;

(ii) a polypeptide which is at least 75% identical in amino acid sequence to SEQ ID NO: 20 or to the polypeptide encoded by SEQ ID NO: 19;

(iii) a polypeptide of SEQ ID NO: 20; and

- 5 (iv) a polypeptide as defined in (i), (ii) or (iii) which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i), (ii) or (iii); and

- 10 instructions for use in eliciting an immunoprotective response in a mammal.

357. A commercial package comprising at least one polypeptide selected from any one of:

- 15 (i) a polypeptide encoded by a nucleic acid sequence comprising at least 36 consecutive nucleotides from SEQ ID NO: 19;

(ii) a polypeptide which is an immunogenic fragment comprising at least 12 consecutive amino acids from SEQ ID No: 20;

- 20 (iii) a polypeptide as defined in (i) or (ii), which has been modified without loss of immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide of (i) or (ii); and

- 25 instructions for use in eliciting an immunoprotective response in a mammal.

358. Expression plasmid pCACPNM708 as shown in Figure 30.

359. A vaccine comprising a vaccine vector and at least one first nucleic acid selected from:

(i) a nucleic acid encoding a polypeptide of any one of SEQ ID Nos: 72 to 74; and

(ii) a nucleic acid sequence as defined in (i) which has been modified to encode a modified conservatively substituted polypeptide, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).

360. A vaccine comprising a vaccine vector and at least one first polypeptide selected from:

(i) a polypeptide of any one of SEQ ID Nos: 72 to 74; and

(ii) a polypeptide as defined in (i) which has been modified by conservative substitution, wherein the modified polypeptide retains immunogenicity and is at least 75% identical in amino acid sequence to the corresponding polypeptide encoded by the nucleic acid of (i).



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**Figure 1. Sequence of *C. pneumoniae* ATP-binding cassette<sup>1</sup> gene (SEQ ID NO: 1 and 2)**

aatcattc	ccccatcgac	taaatccacc	aaggactcgg	acctccatgt	tcttcaatcc	60										
aatatgaacgt	aatattaagt	agcaaattga	gtactatata	atg Met	aag Lys	atg Met	cat His	agg Arg	115							
ctt	aaa	cct	acc	tta	aaa	agt	ctg	atc	cct	aat	ctt	ctt	ttc	tta	ttg	163
Leu	Lys	Pro	Thr	Leu	Lys	Ser	Leu	Ile	Pro	Asn	Leu	Leu	Phe	Leu	Leu	
				10					15					20		
ctc	act	ctt	tca	agc	tcg	tca	aag	caa	aaa	caa	gaa	ccc	tta	gga	aaa	211
Leu	Thr	Leu	Ser	Ser	Cys	Ser	Lys	Gln	Lys	Gln	Glu	Pro	Leu	Gly	Lys	
				25					30				35			
cat	ctc	gtt	att	gcg	atg	agc	cat	gat	ctc	gcc	gac	cta	gat	cct	cgc	259
His	Leu	Val	Ile	Ala	Met	Ser	His	Asp	Leu	Ala	Asp	Leu	Asp	Pro	Arg	
			40					45					50			
aat	gcc	tat	tta	agc	aga	gat	gct	tcc	cta	gca	aaa	gcc	ctc	tat	gaa	307
Asn	Ala	Tyr	Leu	Ser	Arg	Asp	Ala	Ser	Leu	Ala	Lys	Ala	Leu	Tyr	Glu	
						60					65					
gga	ctg	aca	aga	gaa	act	gat	caa	gga	atc	gca	ctg	gct	ctt	gca	gaa	355
Gly	Leu	Thr	Arg	Glu	Thr	Asp	Gln	Gly	Ile	Ala	Leu	Ala	Leu	Ala	Glu	
	70				75				80						85	
agt	tat	acc	ctg	tca	aaa	gat	cat	aag	gtc	tat	acc	ttt	aaa	ctc	aga	403
Ser	Tyr	Thr	Leu	Ser	Lys	Asp	His	Lys	Val	Tyr	Thr	Phe	Lys	Leu	Arg	
				90					95					100		
cct	tct	gtg	tgg	agc	gat	ggc	act	cca	ctc	act	gct	tat	gac	ttt	gaa	451
Pro	Ser	Val	Trp	Ser	Asp	Gly	Thr	Pro	Leu	Thr	Ala	Tyr	Asp	Phe	Glu	
			105					110					115			
aaa	tct	ata	aaa	caa	ctg	tac	ttc	gaa	gaa	ttt	tca	cct	tcc	ata	cat	499
Lys	Ser	Ile	Lys	Gln	Leu	Tyr	Phe	Glu	Glu	Phe	Ser	Pro	Ser	Ile	His	
			120				125					130				
act	tta	ctc	ggc	gtg	att	aaa	aat	tct	tcg	gca	atc	cac	aat	gct	caa	547
Thr	Leu	Leu	Gly	Val	Ile	Lys	Asn	Ser	Ser	Ala	Ile	His	Asn	Ala	Gln	
			135			140					145					
aaa	tct	ctg	gaa	act	ctt	ggg	ata	cag	gca	aaa	gat	gat	ctt	act	ttg	595
Lys	Ser	Leu	Glu	Thr	Leu	Gly	Ile	Gln	Ala	Lys	Asp	Asp	Leu	Thr	Leu	
					155				160						165	
gtg	att	acc	cta	gag	caa	cct	ttc	cca	tac	ttt	ctc	aca	ctt	atc	got	643
Val	Ile	Thr	Leu	Glu	Gln	Pro	Phe	Pro	Tyr	Phe	Leu	Thr	Leu	Ile	Ala	
				170					175					180		

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Figure 1 (Cont.)

cgc ccc gta ttc tcc cct gtt cat cac acc ctt agg gaa tcc tat aag Arg Pro Val Phe Ser Pro Val His His Thr Leu Arg Glu Ser Tyr Lys 185 190 195	691
aaa gga aca ccc cca tcc aca tac atc tcc aat ggg ccc ttt gtc tta Lys Gly Thr Pro Pro Ser Thr Tyr Ile Ser Asn Gly Pro Phe Val Leu 200 205 210	739
aaa aaa cat gaa cac caa aac tac tta att tta gaa aaa aat cct cac Lys Lys His Glu His Gln Asn Tyr Leu Ile Leu Glu Lys Asn Pro His 215 220 225	787
tac tat gat cat gaa tca gta aag tta gac cga gtc acc tta aaa att Tyr Tyr Asp His Glu Ser Val Lys Leu Asp Arg Val Thr Leu Lys Ile 230 235 240 245	835
atc cca gac gcc tcc aca gcc acg aaa ctt ttc aaa agt aaa tct ata Ile Pro Asp Ala Ser Thr Ala Thr Lys Leu Phe Lys Ser Lys Ser Ile 250 255 260	883
gat tgg att ggc tca cct tgg agc gct cgg ata tct aac gaa gac caa Asp Trp Ile Gly Ser Pro Trp Ser Ala Pro Ile Ser Asn Glu Asp Gln 265 270 275	931
aaa gtt ctc tcc caa gaa aag att ctt acc tat tct gtt tca agc acc Lys Val Leu Ser Gln Glu Lys Ile Leu Thr Tyr Ser Val Ser Ser Thr 280 285 290	979
acc ctt ctt atc tat aac ctg caa aaa cct cta ata caa aat aaa gcc Thr Leu Leu Ile Tyr Asn Leu Gln Lys Pro Leu Ile Gln Asn Lys Ala 295 300 305	1027
ctc agg aaa gcc att gct cat gct att gat aga aaa tct atc tta aga Leu Arg Lys Ala Ile Ala His Ala Ile Asp Arg Lys Ser Ile Leu Arg 310 315 320 325	1075
ctc gtg cct tca gga caa gaa gct gta act cta gtt ccc cca aat ctt Leu Val Pro Ser Gly Gln Glu Ala Val Thr Leu Val Pro Pro Asn Leu 330 335 340	1123
tca caa ctc aat ctt caa aaa gag atc tca aca gaa gaa cga caa aca Ser Gln Leu Asn Leu Gln Lys Glu Ile Ser Thr Glu Glu Arg Gln Thr 345 350 355	1171
aaa gcc aga gca tat ttt caa gaa gct aaa gaa aca ctt tct gaa aaa Lys Ala Arg Ala Tyr Phe Gln Glu Ala Lys Glu Thr Leu Ser Glu Lys 360 365 370	1219
gaa ctc gca gaa ctc agc atc ctc tat cct ata gat tcc tcg aat tcc Glu Leu Ala Glu Leu Ser Ile Leu Tyr Pro Ile Asp Ser Ser Asn Ser 375 380 385	1267

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Figure 1 (Cont.)

tcc atc ata gct caa gaa atc caa aga caa ctt aaa gat acc tta gga	1315
Ser Ile Ile Ala Gln Glu Ile Gln Arg Gln Leu Lys Asp Thr Leu Gly	
390 395 400 405	
ttg aaa atc aaa atc caa ggc atg gag tac cac tgc ttt tta aag aaa	1363
Leu Lys Ile Lys Ile Gln Gly Met Glu Tyr His Cys Phe Leu Lys Lys	
410 415 420	
cgt cgt caa gga gat ttc ttc ata gcg aca gga gga tgg att gcg gaa	1411
Arg Arg Gln Gly Asp Phe Phe Ile Ala Thr Gly Gly Trp Ile Ala Glu	
425 430 435	
tac gta agc ccc gta gcc ttc cta tct att cta ggc aac ccc aga gac	1459
Tyr Val Ser Pro Val Ala Phe Leu Ser Ile Leu Gly Asn Pro Arg Asp	
440 445 450	
ctc aca caa tgg aga aac agt gat tac gaa aag act tta gag aaa ctc	1507
Leu Thr Gln Trp Arg Asn Ser Asp Tyr Glu Lys Thr Leu Glu Lys Leu	
455 460 465	
tat ctc cct cat gcc tac aaa gag aat tta aaa cgc gca gaa atg ata	1555
Tyr Leu Pro His Ala Tyr Lys Glu Asn Leu Lys Arg Ala Glu Met Ile	
470 475 480 485	
ata gaa gaa gaa acc ccg att atc ccc ctg tat cac ggc aaa tat att	1603
Ile Glu Glu Glu Thr Pro Ile Ile Pro Leu Tyr His Gly Lys Tyr Ile	
490 495 500	
tac gct ata cat cct aaa atc cag aat aca ttc gga tct ctt cta ggc	1651
Tyr Ala Ile His Pro Lys Ile Gln Asn Thr Phe Gly Ser Leu Leu Gly	
505 510 515	
cac aca gat ctc aaa aat atc gat atc tta agt tagatccgaa atggaaaaat	1704
His Thr Asp Leu Lys Asn Ile Asp Ile Leu Ser	
520 525	
taaaaaat ttt atagacaatc ttgaaaagag aattaaaaat ttttaattta aattatagtt	1764
gcaattgaaa acgcccctaa gaa	1787

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Figure 2. Sequence of *C. pneumoniae* secretory locus ORF gene (SEQ ID NO: 3 and 4).

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ttccagagaa atcctgatcc tgaaaaactt cctgaaacaa ttgctttaac tataacacgg 60
gaacctaag catatcctcc aaggacgtta acataccaat ttg cgg ttg gga aat 115
                                         Leu Arg Leu Gly Asn
                                         1           5

aag cct atg caa cct ttt atc ttt act tta ctg tgc ttg aca tct ttg 163
Lys Pro Met Gln Pro Phe Ile Phe Thr Leu Leu Cys Leu Thr Ser Leu
                                         10           15           20

gtt tct tta gtc gcc ttt gat gct gcg aat gct cgt aaa cgt tgt gcc 211
Val Ser Leu Val Ala Phe Asp Ala Ala Asn Ala Arg Lys Arg Cys Ala
                                         25           30           35

tgt gct caa act ata gaa cgt gga gag aac ttc ttt tcc ata aaa cgc 259
Cys Ala Gln Thr Ile Glu Arg Gly Glu Asn Phe Phe Ser Ile Lys Arg
                                         40           45           50

tct gct tgt gct gaa atc gaa tat caa gaa aaa tct cgc cac gcc tca 307
Ser Ala Cys Ala Glu Ile Glu Tyr Gln Glu Lys Ser Arg His Ala Ser
                                         55           60           65

gca att gaa aga atc tca aaa gat aaa ggc aaa gtc act cca aag cag 355
Ala Ile Glu Arg Ile Ser Lys Asp Lys Gly Lys Val Thr Pro Lys Gln
70           75           80           85

att gcg aaa gta gct act aag aaa aag caa aga tac cgt tta ttg cag 403
Ile Ala Lys Val Ala Thr Lys Lys Lys Gln Arg Tyr Arg Leu Leu Gln
90           95           100

gtt cct ttt tca agg cct ccg aat aac tca agg tat aac ctc tat gct 451
Val Pro Phe Ser Arg Pro Prc Asn Asn Ser Arg Tyr Asn Leu Tyr Ala
105           110           115

ttg ctt agt gaa cct ccc gaa tgc tat agc gat aca gca tca tgg tat 499
Leu Leu Ser Glu Pro Pro Glu Cys Tyr Ser Asp Thr Ala Ser Trp Tyr
120           125           130

gct att ttt att cgg tta ctt cga cgt gct tat gta gac acg gga aat 547
Ala Ile Phe Ile Arg Leu Leu Arg Arg Ala Tyr Val Asp Thr Gly Asn
135           140           145

gta cct cct gga tct gag tat gcc atc gct aat gct ttg ata agt aac 595
Val Pro Pro Gly Ser Glu Tyr Ala Ile Ala Asn Ala Leu Ile Ser Asn
150           155           160           165

aaa caa gag att tta gag agg gga gcg cag ctt gga ccc gat gtt att 643
Lys Gln Glu Ile Leu Glu Arg Gly Ala Gln Leu Gly Pro Asp Val Ile
170           175           180

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Figure 2 (Cont.)

gaa act cta aca ttg cct gag gaa caa gcc gag att ttt tat aaa atg	691
Glu Thr Leu Thr Leu Pro Glu Glu Gln Ala Glu Ile Phe Tyr Lys Met	
185 190 195	
ctc aaa ggg tcg tca aac tct cag tcg cta ctg aat ttt ctg cat tat	739
Leu Lys Gly Ser Ser Asn Ser Gln Ser Leu Leu Asn Phe Leu His Tyr	
200 205 210	
gaa gag aaa agc tta ggc cac tgt aag cta aat ctg atc ttc atg gat	787
Glu Glu Lys Ser Leu Gly His Cys Lys Leu Asn Leu Ile Phe Met Asp	
215 220 225	
ccc cta ctg tta gaa gct gtt cta gat cat ccc gat gct tat agg gaa	835
Pro Leu Leu Leu Glu Ala Val Leu Asp His Pro Asp Ala Tyr Arg Glu	
230 235 240 245	
acg tcg ctc ctg cgc gat ggc att tgg gaa gcg gtg aag cgt caa gaa	883
Thr Ser Leu Leu Arg Asp Gly Ile Trp Glu Ala Val Lys Arg Gln Glu	
250 255 260	
cat gcc atc caa gaa cat ggc cag gca gct gct ttg gag ctt ttt aaa	931
His Ala Ile Gln Glu His Gly Gln Ala Ala Leu Glu Leu Phe Lys	
265 270 275	
aca cgc acc gac ttc cgc ctg gag ctg cga gat aag atg cag tta ctt	979
Thr Arg Thr Asp Phe Arg Leu Glu Leu Arg Asp Lys Met Gln Leu Leu	
280 285 290	
cta agt cga tac gat ttg ctc ccc tta tta aat aaa aaa atg ttc gac	1027
Leu Ser Arg Tyr Asp Leu Leu Pro Leu Leu Asn Lys Lys Met Phe Asp	
295 300 305	
tac acc tta gga agt gcc gga gat tac tta ttt ttg gta gac cca gat	1075
Tyr Thr Leu Gly Ser Ala Gly Asp Tyr Leu Phe Leu Val Asp Pro Asp	
310 315 320 325	
act aag gca att tct cga tgt cgc tgc cct tca aag agt att aaa tta	1123
Thr Lys Ala Ile Ser Arg Cys Arg Cys Pro Ser Lys Ser Ile Lys Leu	
330 335 340	
taatttaatt ttaatatatta ttttaaataag ttttttttga taattgtctt aataagtact	1183
ataaaaaata tttctatagg taggaccatg gcagacgaga ccc	1226

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**Figure 3. Sequence of *C. pneumoniae* Endopeptidase gene (SEQ ID NO: 5 and 6).**

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gttactttttt ttttccataaa aaccccatgt aactttttact tgctcatatt gagaagtccc 60
ccatactata aaaggcaacg ttttcttttc ttgggtttttt atg ctc acc cta ggc 115
                                     Met Leu Thr Leu Gly
                                     1 5
ttg gaa agt tct tgc gat gag act gcc tgc gct ata gtt aat gag gat 163
Leu Glu Ser Ser Cys Asp Glu Thr Ala Cys Ala Ile Val Asn Glu Asp
                                     10 15 20
aag cag ata tta gca aat att att gcc tct caa gat atc cat gca tcc 211
Lys Gln Ile Leu Ala Asn Ile Ile Ile Ser Gln Asp Ile His Ala Ser
                                     25 30 35
tat ggc gga gtc gtt cct gaa ctt gct tca aga gca cat ctc cat atc 259
Tyr Gly Gly Val Val Pro Glu Leu Ala Ser Arg Ala His Leu His Ile
                                     40 45 50
ttc cca caa gtg ata aat aaa gct cta caa cag gcc aac tta ttg atc 307
Phe Pro Gln Val Ile Asn Lys Ala Leu Gln Gln Ala Asn Leu Leu Ile
                                     55 60 65
gaa gat atg gat ctg att gca gta acg caa act cca ggg ttg ata ggt 355
Glu Asp Met Asp Leu Ile Ala Val Thr Gln Thr Pro Gly Leu Ile Gly
                                     70 75 80 85
tct cta tca gta gga gtg cat ttt ggt aaa ggc att gcc ata gga gca 403
Ser Leu Ser Val Gly Val His Phe Gly Lys Gly Ile Ala Ile Gly Ala
                                     90 95 100
aaa aaa tcc ttg att gga gtc aat cac gtc gaa gct cat ctc tat gct 451
Lys Lys Ser Ser Leu Ile Gly Val Asn His Val Glu Ala His Leu Tyr Ala
                                     105 110 115
gcc tat atg gca gcg caa aac gtg caa ttc cct gct tta ggt ctt gtg 499
Ala Tyr Met Ala Ala Gln Asn Val Gln Phe Pro Ala Leu Gly Leu Val
                                     120 125 130
gtc tct gga gct cat acc gca gcg ttt ttt ata gaa aat cct aca tcc 547
Val Ser Gly Ala His Thr Ala Ala Phe Phe Ile Glu Asn Pro Thr Ser
                                     135 140 145
tat aaa ctc ata gga aaa act cga gat gat gct ata gga gaa act ttt 595
Tyr Lys Leu Ile Gly Lys Thr Arg Asp Asp Ala Ile Gly Glu Thr Phe
                                     150 155 160 165
gat aaa gta gga cgc ttt cta gga tta cca tac cct gca ggc cca tta 643
Asp Lys Val Gly Arg Phe Leu Gly Leu Pro Tyr Pro Ala Gly Pro Leu
                                     170 175 180

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Figure 3 (Cont.)

att gaa aaa ctc gct tta gaa ggc tct gag gac agt tat cct ttt agt	691
Ile Glu Lys Leu Ala Leu Glu Gly Ser Glu Asp Ser Tyr Pro Phe Ser	
185 190 195	
cca gct aaa gtc cca aac tat gac ttt tca ttc agc ggt ctt aaa aca	739
Pro Ala Lys Val Pro Asn Tyr Asp Phe Ser Phe Ser Gly Leu Lys Thr	
200 205 210	
gct gtt ctc tac gca atc aaa gga aat aat agt agc ccc cgc tct cct	787
Ala Val Leu Tyr Ala Ile Lys Gly Asn Asn Ser Ser Pro Arg Ser Pro	
215 220 225	
gct cca gag ata tct tta gaa aaa caa aga gat atc gct gct tca ttt	835
Ala Pro Glu Ile Ser Leu Glu Lys Gln Arg Asp Ile Ala Ala Ser Phe	
230 235 240 245	
caa aaa gcg gcc tgc act act att gca caa aaa ctt ccc act att ata	883
Gln-Lys Ala Ala Cys Thr Thr Ile Ala Gln Lys Leu Pro Thr Ile Ile	
250 255 260	
aaa gaa ttt tgc tgc cga tct ata ctt att gga ggt gcc gta gcc att	931
Lys Glu Phe Ser Cys Arg Ser Ile Leu Ile Gly Gly Val Ala Ile	
265 270 275	
aat gaa tac ttt aga tcc gca ata caa act gcg tgt aat cta cct gta	979
Asn Glu Tyr Phe Arg Ser Ala Ile Gln Thr Ala Cys Asn Leu Pro Val	
280 285 290	
tac ttc ccc cct gct aaa cta tgc tca gat aat gct gct atg att gca	1027
Tyr Phe Pro Pro Ala Lys Leu Cys Ser Asp Asn Ala Ala Met Ile Ala	
295 300 305	
ggt cta ggg gga gaa aat ttt caa aaa aac tct agt att ccg gaa att	1075
Gly Leu Gly Gly Glu Asn Phe Gln Lys Asn Ser Ser Ile Pro Glu Ile	
310 315 320 325	
cgt ata tgc gca aga tat cag tgg gaa tct gta tca cca ttc tcc tta	1123
Arg Ile Cys Ala Arg Tyr Gln Trp Glu Ser Val Ser Pro Phe Ser Leu	
330 335 340	
gcc tct ccg tagtcctcca aggctgcaag gagtccagtc actcctctac	1172
Ala Ser Pro	
atctcgggga gaactcgcta ttaataataag agatgaaccc cggtcttttag atocaagaca	1232
agt	1235





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Figure 4 (Cont.)

gcg gtt cct tca gga att gcc atg ttg aaa ctt cgc cga ccc agt ggt	691
Ala Val Pro Ser Gly Ile Ala Met Leu Lys Leu Arg Arg Pro Ser Gly	
185 190 195	
ttg atc cgt tog aca cgg gtc cgt tgg cgt tat act cca gag cat atc	739
Leu Ile Arg Ser Thr Pro Val Arg Trp Arg Tyr Thr Pro Glu His Ile	
200 205 210	
gga gat ttt tct tta gtt gct cct ttg att cct gaa cat aaa cct caa	787
Gly Asp Phe Ser Leu Val Ala Pro Leu Ile Pro Glu His Lys Pro Glu	
215 220 225	
tta cct aca caa agt tgt gtg cta ttc cgt tcc ggg gta aat tca cag	835
Leu Pro Thr Gln Ser Cys Val Leu Phe Arg Ser Gly Val Asn Ser Gln	
230 235 240 245	
tct tct agt agc tct tta ttc agt tcc tac atg gtg cct tat ttc tgg	883
Ser Ser Ser Ser Ser Leu Phe Ser Ser Tyr Met Val Pro Tyr Phe Trp	
250 255 260	
gaa gaa ttg cgg gtt caa aat aag cag cgt ttt gac agt aat cac cat	931
Glu Glu Leu Arg Val Gln Asn Lys Gln Arg Phe Asp Ser Asn His His	
265 270 275	
ata ggg agc cgt aat gga ttt tta cct acg ttt ggt cct att ctt tgg	979
Ile Gly Ser Arg Asn Gly Phe Leu Pro Thr Phe Gly Pro Ile Leu Trp	
280 285 290	
gaa caa gac aag ggg ccc tat cgt tcc tat atc ttt aaa gca aaa gat	1027
Glu Gln Asp Lys Gly Pro Tyr Arg Ser Tyr Ile Phe Lys Ala Lys Asp	
295 300 305	
tct cag ggc aat ccc cat cgc ata gga ttt tta aga att tct tct tat	1075
Ser Gln Gly Asn Pro His Arg Ile Gly Phe Leu Arg Ile Ser Ser Tyr	
310 315 320 325	
gtt tgg act gat tta gaa gga ctt gaa gag gat cat aag gat agt cct	1123
Val Trp Thr Asp Leu Glu Gly Leu Glu Asp His Lys Asp Ser Pro	
330 335 340	
tgg gag ctc ttt gga gag atc atc gat cat ttg gaa aaa gag act gat	1171
Trp Glu Leu Phe Gly Glu Ile Ile Asp His Leu Glu Lys Glu Thr Asp	
345 350 355	
gct ttg att att gat cag acc cat aat cct gga ggc agt gtt ttc tat	1219
Ala Leu Ile Ile Asp Gln Thr His Asn Pro Gly Ser Val Phe Tyr	
360 365 370	
ctc tat tgg tta cta tct atg tta aca gat cat cct tta gat act cct	1267
Leu Tyr Ser Leu Leu Ser Met Leu Thr Asp His Pro Leu Asp Thr Pro	
375 380 385	

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Figure 4 (Cont.)

aaa cat aga atg att ttc act cag gat gaa gtc agc tgc gct ttg cac	1315
Lys His Arg Met Ile Phe Thr Gln Asp Glu Val Ser Ser Ala Leu His	
390 395 400 405	
tggtg caa gat cta cta gaa gat gtc ttc aca gat gag cag gca gtt gcc	1363
Trp Gln Asp Leu Leu Glu Asp Val Phe Thr Asp Glu Gln Ala Val Ala	
410 415 420	
gtg cta ggg gaa act atg gaa gga tat tgc atg gat atg cat gct gta	1411
Val Leu Gly Glu Thr Met Glu Gly Tyr Cys Met Asp Met His Ala Val	
425 430 435	
gcc tct ctt caa aac ttc tct cag agt gtc ctt tct tcc tgg gtt tca	1459
Ala Ser Leu Gln Asn Phe Ser Gln Ser Val Leu Ser Ser Trp Val Ser	
440 445 450	
gggtgat att aac ctt tca aaa cct atg cct ttg cta gga ttt gca cag	1507
Gly Asp Ile Asn Leu Ser Lys Pro Met Pro Leu Leu Gly Phe Ala Gln	
455 460 465	
gttcga cct cat cct aaa cat caa tat act aaa cct ttg ttt atg ttg	1555
Val Arg Pro His Pro Lys His Gln Tyr Thr Lys Pro Leu Phe Met Leu	
470 475 480 485	
ata gac gag gat gac ttc tct tgt gga gat tta gcg cct gca att ttg	1603
Ile Asp Glu Asp Asp Phe Ser Cys Gly Asp Leu Ala Pro Ala Ile Leu	
490 495 500	
aag gat aat ggc cgc gct act ctc att gga aag cca aca gca gga gct	1651
Lys Asp Asn Gly Arg Ala Thr Leu Ile Gly Lys Pro Thr Ala Gly Ala	
505 510 515	
ggagggt ttt gta ttc caa gtc act ttc cct aac cgt tct gga att aaa	1699
Gly Gly Phe Val Phe Gln Val Thr Phe Pro Asn Arg Ser Gly Ile Lys	
520 525 530	
gggtctt tct tta aca gga tct tta gct gtt agg aaa gat ggt gag ttt	1747
Gly Leu Ser Leu Thr Gly Ser Leu Ala Val Arg Lys Asp Gly Glu Phe	
535 540 545	
att gaa aac tta gga gtg gct cct cat att gat tta gga ttt acc tcc	1795
Ile Glu Asn Leu Gly Val Ala Pro His Ile Asp Leu Gly Phe Thr Ser	
550 555 560 565	
agg gat ttg caa act tcc agg ttt act gat tac gtt gag gca gtg aaa	1843
Arg Asp Leu Gln Thr Ser Arg Phe Thr Asp Tyr Val Glu Ala Val Lys	
570 575 580	
act ata gtt tta act tct ttg tct gag aac gct aag aag agt gaa gag	1891
Thr Ile Val Leu Thr Ser Leu Ser Glu Asn Ala Lys Lys Ser Glu Glu	
585 590 595	

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Figure 4 (Cont.)

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cag act tct ccg caa gag acg cct gaa gtt att cga gtc tct tat ccc 1939
Gln Thr Ser Pro Gln Glu Thr Pro Glu Val Ile Arg Val Ser Tyr Pro
      600                      605                      610

aca acg act tct gct tcg taaacgggac gtaatagaat aatttttatt 1987
Thr Thr Thr Ser Ala Ser
      615

attgctttaa tatgcgcgct tccaatataa gcattgtgaa gcgcgtttca tatgtctttt 2047
atcttttaggt aat
                                     2060

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Figure 5. Sequence of *C. pneumoniae* Metalloprotease gene (SEQ ID NO: 9 and 10).

```

gacgtaatag aataattttt attattgctt taatatgcgc gcttccaata taagcattgt 60
gaagcgcggtt tcataatgtct tttatcttta ggtaattatt atg aga aaa ctt att 115
                                     Met Arg Lys Leu Ile
                                     1                               5

tta tgc aat cct aga gga ttt tgc tct gga gtt gtg cgc gct att caa 163
Leu Cys Asn Pro Arg Gly Phe Cys Ser Gly Val Val Arg Ala Ile Gln
                               10                               15                               20

ggt gta gag gtt gct tta gaa aag tgg gga gct cct atc tat gta aaa 211
Val Val Glu Val Ala Leu Glu Lys Trp Gly Ala Pro Ile Tyr Val Lys
                               25                               30                               35

cat gag att gtt cac aat cgc cat gtt gtt aat gct tta cga gcc aag 259
His Glu Ile Val His Asn Arg His Val Val Asn Ala Leu Arg Ala Lys
                               40                               45                               50

gga gcg atc ttt gtt gaa gaa ctt gtt gat gtt cct gaa ggt gag aga 307
Gly Ala Ile Phe Val Glu Glu Leu Val Asp Val Pro Glu Gly Glu Arg
                               55                               60                               65

gtc att tat tca gct cat gga att cct cct tca gtt aga gct gaa gca 355
Val Ile Tyr Ser Ala His Gly Ile Pro Pro Ser Val Arg Ala Glu Ala
                               70                               75                               80                               85

aaa gcc cgt aag ctt att gat att gat gct acc tgt ggt ttg gtt act 403
Lys Ala Arg Lys Leu Ile Asp Ile Asp Ala Thr Cys Gly Leu Val Thr
                               90                               95                               100

aag gtg cat tct gct gcg aag tta tac gca agt aaa gga tac aaa atc 451
Lys Val His Ser Ala Ala Lys Leu Tyr Ala Ser Lys Gly Tyr Lys Ile
                               105                               110                               115

ata ctg atc ggc cat aag aag cac gtt gag gtg att ggt att gtt gga 499
Ile Leu Ile Gly His Lys Lys His Val Glu Val Ile Gly Ile Val Gly
                               120                               125                               130

gaa gtt cct gaa cac att act gtt gtc gag aag gtt gct gac gtc gag 547
Glu Val Pro Glu His Ile Thr Val Val Glu Lys Val Ala Asp Val Glu
                               135                               140                               145

gcc tta cct ttt agt tct gat aca cct tta ttt tat att act caa acg 595
Ala Leu Pro Phe Ser Ser Asp Thr Pro Leu Phe Tyr Ile Thr Gln Thr
                               150                               155                               160                               165

acg ttg agt ttg gat gat gtt cag gag atc tca tcg gct ttg cta aag 643
Thr Leu Ser Leu Asp Asp Val Gln Glu Ile Ser Ser Ala Leu Leu Lys
                               170                               175                               180

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Figure 5 (Cont.)

cga tat ccc tct atc att act ctg cct agt tct tgg att tgt tat gca	691
Arg Tyr Pro Ser Ile Ile Thr Leu Pro Ser Ser Ser Ile Cys Tyr Ala	
185 190	
acc acg aac cgt caa aaa gca ttg cgt tct gtt tta tct cgc gtg aat	739
Thr Thr Asn Arg Gln Lys Ala Leu Arg Ser Val Leu Ser Arg Val Asn	
200 205 210	
tac gtc tat gtg gtt gga gat gtc aac agc tcg aat tcc aat cgt ctt	787
Tyr Val Tyr Val Val Gly Asp Val Asn Ser Ser Asn Ser Asn Arg Leu	
215 220 225	
cgc gaa gtg gct ttg aga agg gga gtt ccc gct gat ttg atc aac aat	835
Arg Glu Val Ala Leu Arg Arg Gly Val Pro Ala Asp Leu Ile Asn Asn	
230 235 240 245	
ccc gag gat att gat acg aac atc gta aat cat tct gga gat ata gca	883
Pro Glu Asp Ile Asp Thr Asn Ile Val Asn His Ser Gly Asp Ile Ala	
250 255 260	
atg act gca gga gcc tca act ccc gaa gac gta gtt caa gct tgc att	931
Met Thr Ala Gly Ala Ser Thr Pro Glu Asp Val Val Gln Ala Cys Ile	
265 270 275	
cga aag cta tca tca ctt atc cct ggt tta caa gtg gaa aat gat ata	979
Arg Lys Leu Ser Ser Leu Ile Pro Gly Leu Gln Val Glu Asn Asp Ile	
280 285 290	
ttt gct gta gag gat gtc gta ttt caa tta cca aaa gaa ctc cgt tgt	1027
Phe Ala Val Glu Asp Val Val Phe Gln Leu Pro Lys Glu Leu Arg Cys	
295 300 305	
tct taggtcttta ggettaacttg ccaagttttt ctcgagattg ctttatagag	1080
Ser	
310	
tctttctctc gtccagagag ggtatttacc tttttagttc tctgtatttg aaa	1133

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**Figure 6. Sequence of *C. pneumoniae* CLP protease ATPase gene (SEQ ID NOS 11 and 12).**

```

catgggagcc gaggaagcca tctcctacgg acttattgat aaggtggtaa cttctgcgaa 60
agaaactaat aaggatacaa gtagcactta gagagaacat atg aat aaa aaa aat 115
                                         Met Asn Lys Lys Asn
                                         1           5

cta act att tgt tca ttt tgc ggt cgg tct gaa aaa gat gta gag aaa 163
Leu Thr Ile Cys Ser Phe Cys Gly Arg Ser Glu Lys Asp Val Glu Lys
                        10           15           20

ctg att gct ggg cct tcg gta tac att tgt gac tac tgc atc aaa tta 211
Leu Ile Ala Gly Pro Ser Val Tyr Ile Cys Asp Tyr Cys Ile Lys Leu
                        25           30           35

tgc tct gga att tta gat aag aaa ccc tcc tct aca ata tcc tca got 259
Cys Ser Gly Ile Leu Asp Lys Lys Pro Ser Ser Thr Ile Ser Ser Ala
                        40           45           50

cca gtt tct gaa aca cct tca cag cct tct gat ctc agg gtg ctt acc 307
Pro Val Ser Glu Thr Pro Ser Gln Pro Ser Asp Leu Arg Val Leu Thr
                        55           60           65

cct aag gaa atc aaa aag cat att gat gaa tat gtc att ggt cag gaa 355
Pro Lys Glu Ile Lys Lys His Ile Asp Glu Tyr Val Ile Gly Gln Glu
                        70           75           80           85

aga gct aaa aag aca atc gct gtt gct gtt tat aat cac tat aaa cgt 403
Arg Ala Lys Lys Thr Ile Ala Val Ala Val Tyr Asn His Tyr Lys Arg
                        90           95           100

ata cgt gct cta cta cat aac aaa cag gta agc tac ggg aaa tct aac 451
Ile Arg Ala Leu Leu His Asn Lys Gln Val Ser Tyr Gly Lys Ser Asn
                        105           110           115

gtg ctt ctc cta ggc cct aca gga tct gga aaa aca tta att gca aaa 499
Val Leu Leu Leu Gly Pro Thr Gly Ser Gly Lys Thr Leu Ile Ala Lys
                        120           125           130

aca ttg gca aaa att tta gat gtt ccc ttc acc ata gcc gac gca acg 547
Thr Leu Ala Lys Ile Leu Asp Val Pro Phe Thr Ile Ala Asp Ala Thr
                        135           140           145

acc cta acg gaa gca ggt tat gtc ggt gaa gat gta gag aac att gtc 595
Thr Leu Thr Glu Ala Gly Tyr Val Gly Glu Asp Val Glu Asn Ile Val
                        150           155           160           165

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Figure 6 (Cont.)

tta cgt tta tta caa gct gct gat tac gat gtc gcc cgt gca gaa cga	643
Leu Arg Leu Leu Gln Ala Ala Asp Tyr Asp Val Ala Arg Ala Glu Arg	
170 175 180	
ggc att atc tat atc gat gaa atc gat aaa att gga agg aca aca gca	691
Gly Ile Ile Tyr Ile Asp Glu Ile Asp Lys Ile Gly Arg Thr Thr Ala	
185 190 195	
aac gtc tcc att act aga gat gtt tct ggc gaa ggg gtt caa caa gca	739
Asn Val Ser Ile Thr Arg Asp Val Ser Gly Glu Gly Val Gln Gln Ala	
200 205 210	
ttg tta aaa atc gtt gaa gga acc aca gca aac gtt cct cct aaa gga	787
Leu Leu Lys Ile Val Glu Gly Thr Thr Ala Asn Val Pro Pro Lys Gly	
215 220 225	
gga cgt aag cat cct aac caa gag tat atc cga gtc aat acg gaa aat	835
Gly Arg Lys His Pro Asn Gln Glu Tyr Ile Arg Val Asn Thr Glu Asn	
230 235 240 245	
atc tta ttt atc gta ggc gga gcc ttc gtc aac cta gat aag att atc	883
Ile Leu Phe Ile Val Gly Gly Ala Phe Val Asn Leu Asp Lys Ile Ile	
250 255 260	
gca aag cga ttg ggg aaa act acc ata ggg ttt tct gat gat caa gca	931
Ala Lys Arg Leu Gly Lys Thr Thr Ile Gly Phe Ser Asp Asp Gln Ala	
265 270 275	
gac ctc tct caa aaa acc aga gac cat cta ctt gct aaa gtt gaa acc	979
Asp Leu Ser Gln Lys Thr Arg Asp His Leu Leu Ala Lys Val Glu Thr	
280 285 290	
gaa gac ctg att gcc ttc gga atg atc cct gaa ttt gtc gga aga ttc	1027
Glu Asp Leu Ile Ala Phe Gly Met Ile Pro Glu Phe Val Gly Arg Phe	
295 300 305	
aac tgc att gta aac tgt gaa gag ctt tct ttg gat gag ctt gta gcc	1075
Asn Cys Ile Val Asn Cys Glu Glu Leu Ser Leu Asp Glu Leu Val Ala	
310 315 320 325	
atc ctt aca gaa cct aca aat gog att gtg aaa caa tat atg gag cta	1123
Ile Leu Thr Glu Pro Thr Asn Ala Ile Val Lys Gln Tyr Met Glu Leu	
330 335 340	
ttc gca gaa gaa aac gtc aag tta gtc ttc aaa aaa gaa gcc cta tat	1171
Phe Ala Glu Glu Asn Val Lys Leu Val Phe Lys Lys Glu Ala Leu Tyr	
345 350 355	
gct ata gca aaa aaa gcc aag caa gca aaa act gga gct cgt gct cta	1219
Ala Ile Ala Lys Lys Ala Lys Gln Ala Lys Thr Gly Ala Arg Ala Leu	
360 365 370	

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Figure 6 (Cont.)

ggg atg atc cta gaa aat ctc ctt aga gac ctt atg ttt gaa att cct	1267
Gly Met Ile Leu Glu Asn Leu Leu Arg Asp Leu Met Phe Glu Ile Pro	
375 380 385	
tca gat cct aca gta gaa gct att cat atc caa gaa gac act atc gca	1315
Ser Asp Pro Thr Val Glu Ala Ile His Ile Gln Glu Asp Thr Ile Ala	
390 395 400 405	
gaa aat aaa gcg cca ata att atc aga agg acc cca gaa gct atc gct	1363
Glu Asn Lys Ala Pro Ile Ile Ile Arg Arg Thr Pro Glu Ala Ile Ala	
410 415 420	
tagctctttt tagttcctat tttaggggtg tcatgacaac aattgccata gaagctgcaa	1423
aaaaagttct tatcaaacta cgtaatgcag gatatcaggc ata	1466



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Figure 7: Sequence of *C. pneumoniae* CLP protease subunit gene (SEQ ID NOS: 13 and 14).

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tgacgtagac agcctaaaaa gtcttagcta cgctcctaggt gtcattcgt gatcggaac 60
gtatggacac aactgaaaat tatttgatga ggaaacgcaa atg aca ctg gta ccc 115
                                     Met Thr Leu Val Pro
                                     1 5
tat gtt gtc gag gat acg ggc cgt ggt gaa agg gcc atg gat att tac 163
Tyr Val Val Glu Asp Thr Gly Arg Gly Glu Arg Ala Met Asp Ile Tyr
                                     10 15 20
tcc cgt ctt ctg aaa gat cgt att gta atg atc ggt cag gaa atc acg 211
Ser Arg Leu Leu Lys Asp Arg Ile Val Met Ile Gly Gln Glu Ile Thr
                                     25 30 35
gag ccc ctc gca aac aca gta att gcc cag ctc ctt ttc ctc atg tcc 259
Glu Pro Leu Ala Asn Thr Val Ile Ala Gln Leu Leu Phe Leu Met Ser
                                     40 45 50
gaa gat cct aaa aag gat att caa att ttc atc aat tcc cca ggc ggc 307
Glu Asp Pro Lys Lys Asp Ile Gln Ile Phe Ile Asn Ser Pro Gly Gly
                                     55 60 65
tac atc acc gct gga ctg gca atc tat gat acc att cgc ttt tta ggt 355
Tyr Ile Thr Ala Gly Leu Ala Ile Tyr Asp Thr Ile Arg Phe Leu Gly
                                     70 75 80 85
tgt gat gta aat acc tac tgc atc ggt caa gct gca tcc atg gga gcc 403
Cys Asp Val Asn Thr Tyr Cys Ile Gly Gln Ala Ala Ser Met Gly Ala
                                     90 95 100
ctc tta tta tcc gca gga act aaa gga aag cgt cac gct ctt ccc cat 451
Leu Leu Leu Ser Ala Gly Thr Lys Gly Lys Arg His Ala Leu Pro His
                                     105 110 115
agc cgt atg atg atc cac caa cct tct gga ggc att atc gga aca tcc 499
Ser Arg Met Met Ile His Gln Pro Ser Gly Gly Ile Ile Gly Thr Ser
                                     120 125 130
gca gac atc caa ctc caa gca gct gaa att cta aca cta aaa aaa cac 547
Ala Asp Ile Gln Leu Gln Ala Ala Glu Ile Leu Thr Leu Lys Lys His
                                     135 140 145
ctt gcc aat atc ctc tct gaa tgc aca gga caa cct gta gaa aaa att 595
Leu Ala Asn Ile Leu Ser Glu Cys Thr Gly Gln Pro Val Glu Lys Ile
                                     150 155 160 165

```

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Figure 7 (Cont.)

ata gaa gat tct gaa cga gat ttc ttc atg gga gcc gag gaa gcc atc	643
Ile Glu Asp Ser    Glu Arg Asp Phe    Phe Met Gly Ala Glu Glu Ala Ile	
170                  175                  180	
tcc tac gga ctt att gat aag gtg gta act tct gcg aaa gaa act aat	691
Ser Tyr Gly Leu Ile Asp Lys Val Val Thr Ser Ala Lys Glu Thr Asn	
185                  190                  195	
aag gat aca agt agc act tagagagaac atatgaataa aaaaatctta	739
Lys Asp Thr Ser Ser Thr	
200	
actatttggt cattttgcgg tcggtotgaa aaagatgtag agaaactgat tgotgggcoct	799
tcggtatata ttt	812

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**Figure 8: Sequence of *C. pneumoniae* transglycolase/ transpeptidase gene (SEQ ID NOS: 15 and 16).**

gataaaatag aaagacotga tcatttggatg gaaatagcag ctcttccoga ataccaatat																60
ttgggaatac cctcagaaga aagtatcagt cttttatcct																115
Met Ser Tyr Arg Lys 1 5																
cgt tcg act cta att gtt cta gga gtg ttt gct ctt tat gct ott cta																163
Arg Ser Thr Leu Ile Val Leu Gly Val Phe Ala Leu Tyr Ala Leu Leu 10 15 20																
gta ttg cgt tat tat aaa att caa att gaa gga gac cac tgg gcc																211
Val Leu Arg Tyr Tyr Lys Ile Gln Ile Cys Glu Gly Asp His Trp Ala 25 30 35																
gca gaa gct ctc ggg caa cac gaa ttt tgt gtc cgt gat cct ttt cga																259
Ala Glu Ala Leu Gly Gln His Glu Phe Cys Val Arg Asp Pro Phe Arg 40 45 50																
agg gcc acc ttt ttt gct aac acg aca gta cgt aag gga gac aaa gac																307
Arg Gly Thr Phe Phe Ala Asn Thr Thr Val Arg Lys Gly Asp Lys Asp 55 60																
ctt cag cag cct ttc gct gtc gat att aca aaa ttt cac ctt tgt gca																355
Leu Gln Gln Pro Phe Ala Val Asp Ile Thr Lys Phe His Leu Cys Ala 70 75 80 85																
gat cct tta gct att ccc gaa tgt cat cgt gat gag atc atc caa ggg																403
Asp Pro Leu Ala Ile Pro Glu Cys His Arg Asp Glu Ile Ile Gln Gly 90 95 100																
att ctc caa ttt att gag ggg cag acc tac gac gac ctc tcc cta aag																451
Ile Leu Gln Phe Ile Glu Gly Gln Thr Tyr Asp Asp Leu Ser Leu Lys 105 110 115																
tta gat aag aaa tct cgg tat tgt aag ctg tat cct tta tta gat gtt																499
Leu Asp Lys Lys Ser Arg Tyr Cys Lys Leu Tyr Pro Leu Leu Asp Val 120 125 130																
tct gtc cat gac ogg cta tcc ctt tgg tgg aaa gga tat gca aca aag																547
Ser Val His Asp Arg Leu Ser Leu Trp Trp Lys Gly Tyr Ala Thr Lys 135 140 145																
cat cgc tta cca aca aac gcc cta ttt ttt att acg gac tac caa cgc																595
His Arg Leu Pro Thr Asn Ala Leu Phe Phe Ile Thr Asp Tyr Gln Arg 150 155 160 165																

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Figure 8 (Cont.)

tcg tat cct ttt ggg aag ctc ctt gga caa gtt ctc cat acc tta aga	643
Ser Tyr Pro Phe Gly Lys Leu Leu Gly Gln Val Leu His Thr Leu Arg	
170 175 180	
gaa att aag gat gag aaa aca gga aaa gcc ttt ccc aca ggc ggg atg	691
Glu Ile Lys Asp Glu Lys Thr Gly Lys Ala Phe Pro Thr Gly Gly Met	
185 190 195	
gag gcg tac ttt aat cat att ctc gaa ggg gac gtt gga gag aga aag	739
Glu Ala Tyr Phe Asn His Ile Leu Glu Gly Asp Val Gly Glu Arg Lys	
200 205 210	
ctg ttg cgt tct cct ttg aac cgt tta gat acg aat cgt gtt atc aaa	787
Leu Leu Arg Ser Pro Leu Asn Arg Leu Asp Thr Asn Arg Val Ile Lys	
215 220 225	
ctg cct aaa gat ggc tct gat atc tac ctt acg atc aat cct gtg atc	835
Leu Pro Lys Asp Gly Ser Asp Ile Tyr Leu Thr Ile Asn Pro Val Ile	
230 235 240 245	
cag acc att gca gag gaa gaa ctc gaa cgg ggc gtg cta gaa gct aaa	883
Gln Thr Ile Ala Glu Glu Glu Leu Glu Arg Gly Val Leu Glu Ala Lys	
250 255 260	
gcc cag ggg ggt agg ctc att cta atg aac tcc caa aca gga gag att	931
Ala Gln Gly Gly Arg Leu Ile Leu Met Asn Ser Gln Thr Gly Glu Ile	
265 270 275	
ctt gca ctg gct caa tat ccg ttt ttc gat ccc aca aat tat aag gaa	979
Leu Ala Leu Ala Gln Tyr Pro Phe Phe Asp Pro Thr Asn Tyr Lys Glu	
280 285 290	
tac ttc aat aac aaa gag cgc atc gaa cat acg aag gta tct ttt gtg	1027
Tyr Phe Asn Asn Lys Glu Arg Ile Glu His Thr Lys Val Ser Phe Val	
295 300 305	
agc gat gtt ttt gaa ccc ggg tcg atc atg aaa cct ttg act gtg gcg	1075
Ser Asp Val Phe Glu Pro Gly Ser Ile Met Lys Pro Leu Thr Val Ala	
310 315 320 325	
att gct tta caa gct aac gaa gag gct agc tta aaa tcg cag aaa aag	1123
Ile Ala Leu Gln Ala Asn Glu Glu Ala Ser Leu Lys Ser Gln Lys Lys	
330 335 340	
att ttt gat cct gaa gaa cct atc gat gtg acc agg aca ctc ttc cct	1171
Ile Phe Asp Pro Glu Glu Pro Ile Asp Val Thr Arg Thr Leu Phe Pro	
345 350 355	
gga cga aaa gga tct ccg ctt aag gat att tct aga aac tct caa ttg	1219
Gly Arg Lys Gly Ser Pro Leu Lys Asp Ile Ser Arg Asn Ser Gln Leu	
360 365 370	

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Figure 8 (Cont.)

aat atg tac atg gct atc cag aaa tct tcg aat gtc tat gta gct cag	1267
Asn Met Tyr Met Ala Ile Gln Lys Ser Ser Asn Val Tyr Val Ala Gln	
375 380 385	
ctg gct gac cgc atc ata caa tct tta gga gtg gcc tgg tac caa cag	1315
Leu Ala Asp Arg Ile Ile Gln Ser Leu Gly Val Ala Trp Tyr Gln Gln	
390 395 400 405	
aag ttg cta gct ctg gga ttt gga aga aaa aca ggg atc gag ctt ccc	1363
Lys Leu Leu Ala Leu Gly Phe Gly Arg Lys Thr Gly Ile Glu Leu Pro	
410 415 420	
agt gag gcc tct ggt ttg gtg cct tct ccc cat cgt ttc cat att aat	1411
Ser Glu Ala Ser Gly Leu Val Pro Ser Pro His Arg Phe His Ile Asn	
425 430 435	
ggt tcc ctg gaa tgg tcc tta tct act cca tat tct ttg gct atg gga	1459
Gly Ser Leu Glu Trp Ser Leu Ser Thr Pro Tyr Ser Leu Ala Met Gly	
440 445 450	
tat aat att ttg gca aca ggg ata caa atg gtt caa gcc tac gct atc	1507
Tyr Asn Ile Leu Ala Thr Gly Ile Gln Met Val Gln Ala Tyr Ala Ile	
455 460 465	
ctt gca aac gga ggt tat gcc gtc cgg ccc act tta gta aaa aag atc	1555
Leu Ala Asn Gly Gly Tyr Ala Val Arg Pro Thr Leu Val Lys Lys Ile	
470 475 480 485	
gtc tct gct tca gga gag gaa tat cat ctt cct act aaa gag aag aca	1603
Val Ser Ala Ser Gly Glu Glu Tyr His Leu Pro Thr Lys Glu Lys Thr	
490 495 500	
cga ctc ttt tca gaa gaa att act aga gaa gtt gtt cgt gcc atg cgt	1651
Arg Leu Phe Ser Glu Glu Ile Thr Arg Glu Val Val Arg Ala Met Arg	
505 510 515	
ttt aca acg tta ccc gga ggt tcg gga ttt cga gcc tct cct aag cat	1699
Phe Thr Thr Leu Pro Gly Gly Ser Gly Phe Arg Ala Ser Pro Lys His	
520 525 530	
cac tct agt gct ggg aaa aca gga act aca gaa aag atg att cat gga	1747
His Ser Ser Ala Gly Lys Thr Gly Thr Thr Glu Lys Met Ile His Gly	
535 540 545	
aaa tat gat aaa cgc cgt cat att gct tct ttt ata ggt ttt act ccc	1795
Lys Tyr Asp Lys Arg Arg His Ile Ala Ser Phe Ile Gly Phe Thr Pro	
550 555 560 565	
gta gag agc tcg gag gga aat ttc cca cct tta gtg atg ctc gtc tcc	1843
Val Glu Ser Ser Glu Gly Asn Phe Pro Pro Leu Val Met Leu Val Ser	
570 575 580	

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Figure 8 (Cont.)

ata gat gat cct gaa tat ggt ttg cga gcc gac ggc acg aaa aat tat	1891
Ile Asp Asp Pro Glu Tyr Gly Leu Arg Ala Asp Gly Thr Lys Asn Tyr	
585 590 595	
atg ggg ggg cgt tgt gcg gca ccc att ttt tct agg gtt gct gac cgc	1939
Met Gly Gly Arg Cys Ala Ala Pro Ile Phe Ser Arg Val Ala Asp Arg	
600 605 610	
aca ctc ctc tat tta ggg att ott cca gac aag aag cta aga aat tgc	1987
Thr Leu Leu Tyr Leu Gly Ile Leu Pro Asp Lys Lys Leu Arg Asn Cys	
615 620 625	
gac gaa gaa gct gct gca tta aag cgt ctc tat gaa gaa tgg aat cgt	2035
Asp Glu Glu Ala Ala Ala Leu Lys Arg Leu Tyr Glu Glu Trp Asn Arg	
630 635 640 645	
tct ccg aaa caa ggg gga acg agg tgaggatctc tatttccato ttgctataga	2089
Ser Pro Lys Gln Gly Gly Thr Arg	
650	
cttttacggt tgagcaaaga ctctctatca gagagcccggt ctctcttita tctctatga	2149
gtagtttatg tta	2162

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Figure 9: Sequence of *C. pneumoniae* CLPc protease gene (SEQ ID NOS: 17 and 18).

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gaattttacc aaatttgctg gtttagagcg aagagttgca tcattatttt aaatttcgta 60
tatgcttaag gaaagtctta cccctgtctt ttagggtttt atg ttt gag aag ttc 115
Met Phe Glu Lys Phe
1 5
act aat aga gca aaa caa gtc att aaa ctg gcg aaa aag gag gct cag 163
Thr Asn Arg Ala Lys Gln Val Ile Lys Leu Ala Lys Lys Glu Ala Gln
10 15 20
cgt tta aat cat aac tac ctg ggt act gag cac atc ctg ctt ggt ctt 211
Arg Leu Asn His Asn Tyr Leu Gly Thr Glu His Ile Leu Leu Gly Leu
25 30 35
ctc aaa ctt ggt caa ggg gta gct gtt aat gta tta cgc aac ctc ggt 259
Leu Lys Leu Gly Gln Gly Val Ala Val Asn Val Leu Arg Asn Leu Gly
40 45 50
ata gat ttt gat acg gca cgg caa gag gtg gaa cgc ctg att ggt tat 307
Ile Asp Phe Asp Thr Ala Arg Gln Glu Val Glu Arg Leu Ile Gly Tyr
55 60 65
ggt cca gaa att caa gtc tac gga gac cct gcc ctt aca gga aga gta 355
Gly Pro Glu Ile Gln Val Tyr Gly Asp Pro Ala Leu Thr Gly Arg Val
70 75 80 85
aaa aaa tct ttt gaa tca gca aat gaa gag gcc agc ctt tta gag cac 403
Lys Lys Ser Phe Glu Ser Ala Asn Glu Glu Ala Ser Leu Leu Glu His
90 95 100
aat tat gtc ggg acg gag cat tta ctc tta ggg atc cta cat caa tca 451
Asn Tyr Val Gly Thr Glu His Leu Leu Gly Ile Leu His Gln Ser
105 110 115
gat agt gtc gct ctt cag gta tta gaa aac tta cat atc gat cca aga 499
Asp Ser Val Ala Leu Gln Val Leu Glu Asn Leu His Ile Asp Pro Arg
120 125 130
gag gtt cgt aag gaa att ctt aga gaa tta gag acc ttc aat cta caa 547
Glu Val Arg Lys Glu Ile Leu Arg Glu Leu Glu Thr Phe Asn Leu Gln
135 140 145
ctt cct cct tcg tcg tcg tct tct tcc tca tcc tct cga agc aac cct 595
Leu Pro Pro Ser Ser Ser Ser Ser Ser Ser Ser Arg Ser Asn Pro
150 155 160 165
tca tct tca aaa tct cct tta ggt cat agc tta ggt tct gac aaa aac 643
Ser Ser Ser Lys Ser Pro Leu Gly His Ser Leu Gly Ser Asp Lys Asn
170 175 180

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Figure 9 (Cont.)

gaa aag ctt tct gct ctg aaa gca tat ggt tat gat tta acg gag atg Glu Lys Leu Ser Ala Leu Lys Ala Tyr Gly Tyr Asp Leu Thr Glu Met 185 190 195	691
gtc cga gag tct aag ctc gat cct gtc att ggt cgt tct tca gaa gtc Val Arg Glu Ser Lys Leu Asp Pro Val Ile Gly Arg Ser Ser Glu Val 200 205 210	739
gaa cgg ttg att ttg att ctt tgc cga aga aga aaa aac aat cct gta Glu Arg Leu Ile Leu Ile Leu Cys Arg Arg Arg Lys Asn Asn Pro Val 215 220 225	787
ctt att gga gaa gct gga gtt ggt aag act gca att gtt gag ggt ctg Leu Ile Gly Glu Ala Gly Val Gly Lys Thr Ala Ile Val Glu Gly Leu 230 235 240 245	835
gct caa aaa atc att ctg aat gag gtt cct gat gcc tta cgg aaa aag Ala Gln Lys Ile Ile Leu Asn Glu Val Pro Asp Ala Leu Arg Lys Lys 250 255 260	883
cga ctg att act cta gat cta gca tta atg att gct gga aca aaa tat Arg Leu Ile Thr Leu Asp Leu Ala Leu Met Ile Ala Gly Thr Lys Tyr 265 270 275	931
cga ggg caa ttt gag gaa cgg atc aaa gct gtc atg gat gaa gtt cgc Arg Gly Gln Phe Glu Glu Arg Ile Lys Ala Val Met Asp Glu Val Arg 280 285 290	979
aag cat gga aac atc ttg ctc ttc att gac gag ctc cac acg att gta Lys His Gly Asn Ile Leu Leu Phe Ile Asp Glu Leu His Thr Ile Val 295 300 305	1027
gga gca gga gca gct gaa ggt gct atc gat gct tca aac att tta aaa Gly Ala Gly Ala Ala Glu Gly Ala Ile Asp Ala Ser Asn Ile Leu Lys 310 315 320 325	1075
cct gcg tta gcg cga ggt gaa att cag tgt att gga gca act acg ata Pro Ala Leu Ala Arg Gly Glu Ile Gln Cys Ile Gly Ala Thr Thr Ile 330 335 340	1123
gat gag tat cgc aag cac ata gaa aaa gac gca gct tta gaa cgt cgt Asp Glu Tyr Arg Lys His Ile Glu Lys Asp Ala Ala Leu Glu Arg Arg 345 350 355	1171
ttc caa aaa atc gtg gtt cac cct cct agt gta gat gag act att gag Phe Gln Lys Ile Val Val His Pro Pro Ser Val Asp Glu Thr Ile Glu 360 365 370	1219
att tta cgt ggc ctc aag aaa aag tat gaa gaa cat cac aat gtc ttc Ile Leu Arg Gly Leu Lys Lys Lys Tyr Glu Glu His His Asn Val Phe 375 380 385	1267



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Figure 9 (Cont.)

att act gaa gaa gct tta aaa gca gct gcg act ctt tct gat caa tat	1315
Ile Thr Glu Glu Ala Leu Lys Ala Ala Ala Thr Leu Ser Asp Gln Tyr	
390 395 400 405	
gtt cat gga cgt ttc ctc cct gat aaa gca ata gat ctt tta gat gaa	1363
Val His Gly Arg Phe Leu Pro Asp Lys Ala Ile Asp Leu Leu Asp Glu	
410 415 420	
gct ggg gct cgt gtc cgt gtg aat aca atg ggt cag cct aca gat tta	1411
Ala Gly Ala Arg Val Arg Val Asn Thr Met Gly Gln Pro Thr Asp Leu	
425 430 435	
atg aag cta gag gct gaa atc gaa aat aca aaa ttg gcc aaa gag cag	1459
Met Lys Leu Glu Ala Glu Ile Glu Asn Thr Lys Leu Ala Lys Glu Gln	
440 445 450	
gcc att gga act caa gaa tac gaa aaa gct gca ggt tta cgt gat gaa	1507
Ala Ile Gly Thr Gln Glu Tyr Glu Lys Ala Ala Gly Leu Arg Asp Glu	
455 460 465	
gag aaa aaa ctt cgc gaa cgt ctg caa agt atg aaa cag gaa tgg gaa	1555
Glu Lys Lys Leu Arg Glu Arg Leu Gln Ser Met Lys Gln Glu Trp Glu	
470 475 480 485	
aat cat aaa gaa gag cac caa gtt cct gta gat gaa gaa gca gtc gct	1603
Asn His Lys Glu Glu His Gln Val Pro Val Asp Glu Glu Ala Val Ala	
490 495 500	
cag gta gtt tct cta caa aca gga att ccc tca gca agg ctc aca gaa	1651
Gln Val Val Ser Leu Gln Thr Gly Ile Pro Ser Ala Arg Leu Thr Glu	
505 510 515	
gct gaa agt gag aag ctt ctg aag tta gaa gac acg tta aga aga aaa	1699
Ala Glu Ser Glu Lys Leu Leu Lys Leu Glu Asp Thr Leu Arg Arg Lys	
520 525 530	
gtc att ggt caa aat gat gcc gtt acc agc att tgc cgt gcc atc cga	1747
Val Ile Gly Gln Asn Asp Ala Val Thr Ser Ile Cys Arg Ala Ile Arg	
535 540 545	
cgt tct cga aca ggg atc aaa gat cct aac cga cct acg ggc tcc ttc	1795
Arg Ser Arg Thr Gly Ile Lys Asp Pro Asn Arg Pro Thr Gly Ser Phe	
550 555 560 565	
cta ttc ctt ggg cct acc ggt gta ggg aaa agc ctg ctc gcc caa caa	1843
Leu Phe Leu Gly Pro Thr Gly Val Gly Lys Ser Leu Leu Ala Gln Gln	
570 575 580	
att gct ata gag atg ttc ggt ggt gaa gac gct ctg att cag gta gac	1891
Ile Ala Ile Glu Met Phe Gly Gly Glu Asp Ala Leu Ile Gln Val Asp	
585 590 595	

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Figure 9 (Cont.)

atg tca gag tac atg gag aaa ttt gct gct acc aag atg atg gga tca	1939
Met Ser Glu Tyr Met Glu Lys Phe Ala Ala Thr Lys Met Met Gly Ser	
600 605 610	
cct cca gga tat gta ggt cat gaa gaa ggg ggc cac ctt acg gaa cag	1987
Pro Pro Gly Tyr Val Gly His Glu Glu Gly Gly His Leu Thr Glu Gln	
615 620 625	
gta cgt cgc cgt cct tac tgc gtt gtt ctc ttt gat gag ata gaa aag	2035
Val Arg Arg Arg Pro Tyr Cys Val Val Leu Phe Asp Glu Ile Glu Lys	
630 635 640 645	
gca cac cca gac att atg gac ctg atg ttg caa att tta gag caa gga	2083
Ala His Pro Asp Ile Met Asp Leu Met Leu Gln Ile Leu Glu Gln Gly	
650 655 660	
cgt ctt act gat tct ttt ggt cgc aaa gtg gat ttc cgt cat gcc att	2131
Arg Leu Thr Asp Ser Phe Gly Arg Lys Val Asp Phe Arg His Ala Ile	
665 670 675	
att atc atg acc tcc aat ttg gga gct gat ctc att cgt aaa agc gga	2179
Ile Ile Met Thr Ser Asn Leu Gly Ala Asp Leu Ile Arg Lys Ser Gly	
680 685 690	
gaa att ggt ttt ggc ttg aag tcc cat atg gac tat aag gtc atc caa	2227
Glu Ile Gly Phe Gly Leu Lys Ser His Met Asp Tyr Lys Val Ile Gln	
695 700 705	
gag aaa atc gaa cat gct atg aag aaa cac tta aag cct gag ttc att	2275
Glu Lys Ile Glu His Ala Met Lys Lys His Leu Lys Pro Glu Phe Ile	
710 715 720 725	
aac cgt ttg gat gaa agt gtg att ttc cgt ccc ctc gag aaa gaa tct	2323
Asn Arg Leu Asp Glu Ser Val Ile Phe Arg Pro Leu Glu Lys Glu Ser	
730 735 740	
cta tcg gag atc atc cat tta gag atc aac aaa ctg gac tcg aga ctg	2371
Leu Ser Glu Ile Ile His Leu Glu Ile Asn Lys Leu Asp Ser Arg Leu	
745 750 755	
aaa aac tac caa atg gct ttg aac atc coa gac tct gtg att tcc ttc	2419
Lys Asn Tyr Gln Met Ala Leu Asn Ile Pro Asp Ser Val Ile Ser Phe	
760 765 770	
cta gta acg aag ggg cat tct cca gaa atg gga gca cgt cct cta cgc	2467
Leu Val Thr Lys Gly His Ser Pro Glu Met Gly Ala Arg Pro Leu Arg	
775 780 785	
cgt gtc att gag cag tac ctt gaa gat cct cta gcg gag ctc ttg ctt	2515
Arg Val Ile Glu Gln Tyr Leu Glu Asp Pro Leu Ala Glu Leu Leu	
790 795 800 805	

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Figure 9 (Cont.)

aaa gag tcc tgc cgt caa gaa gct cgc aag cta cga gca acc ttg gtt	2563
Lys Glu Ser Cys Arg Gln Glu Ala Arg Lys Leu Arg Ala Thr Leu Val	
810 815 820	
 gaa aat cgc gtt gcc ttt gaa agg gaa gaa gag gag cag gaa gct gct	2611
Glu Asn Arg Val Ala Phe Glu Arg Glu Glu Glu Glu Gln Glu Ala Ala	
825 830 835	
 ctc cct agc cct cac ttg gaa tca taggaacgtc gataactcca ctaccaaggc	2665
Leu Pro Ser Pro His Leu Glu Ser	
840 845	
 aggtatctcc ttgataaaac gctattgttt gtccctggagt taccgccttg acgggttgtg	2725
aaaatcgcac ctt	2738

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Figure 10: Sequence of *C. pneumoniae* Thioredoxin gene (SEQ ID NOS: 19 and 20).

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gattcaggtt ctagtgcgt tatgctcatg gaagttcaag tcttcttagc tgcaagaaaa 60
taacaggggac agtaattcga tttttcgaga agggaaactt atg gta aag atc ata 115
                                     Met Val Lys Ile Ile
                                     1 5

tca agt gaa aat ttt gac tct ttt att gca tgg ggg ctc gtt ctc gtt 163
Ser Ser Glu Asn Phe Asp Ser Phe Ile Ala Ser Gly Leu Val Leu Val
                10                15                20

gat ttc ttt gca gaa tgg tgt ggc ccc tgt cgg atg ctc act cct atc 211
Asp Phe Phe Ala Glu Trp Cys Gly Pro Cys Arg Met Leu Thr Pro Ile
                25                30                35

tta gaa aat ctt gct gcg gaa ctt cct cat gtc act att gga aaa atc 259
Leu Glu Asn Leu Ala Ala Glu Leu Pro His Val Thr Ile Gly Lys Ile
                40                45                50

aat ata gat gag aac agc aag cct gca gaa acg tac gaa gtc agc tct 307
Asn Ile Asp Glu Asn Ser Lys Pro Ala Glu Thr Tyr Glu Val Ser Ser
                55                60                65

att cct acg ctt att ctt ttt aag gat ggg aac gag gtg gct cgg gtc 355
Ile Pro Thr Leu Ile Leu Phe Lys Asp Gly Asn Glu Val Ala Arg Val
                70                75                80                85

gta ggt ctt aag gat aaa gaa ttc cta acc aat ctt atc aat aag cac 403
Val Gly Leu Lys Asp Lys Glu Phe Leu Thr Asn Leu Ile Asn Lys His
                90                95                100

gct taaaaagaag ctgcaatatt aaacgtagg attcttttgc aatgctacgg 456
Ala

ttttctgcct taccacttca tataaaacga tccctacact ggtagctaaa ttt 509

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Figure 11. Restriction enzyme analysis of the *C. pneumoniae* ATP-binding cassette gene (SEQ ID NO: 1).

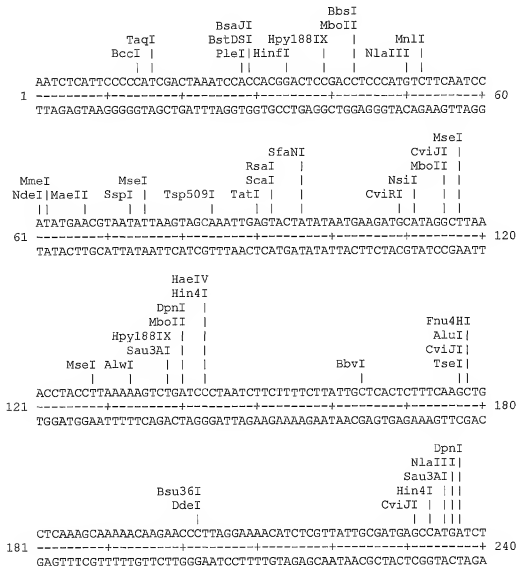


Figure 11 (Cont.)

DpnI  
 BstYI |  
 Sau3AI |  
 BfaI |  
 AlwI |

MseI  
 SfaNI |  
 BsrDI |  
 MnlI |

BfaI  
 MwoI  
 CviJI

241  
 CGCGGACCTAGATCCTCGCAATGCTTATTAAAGCAGAGATGCTTCCTAGCAAAAGCCCT  
 -----  
 GCGGCTGGATCTAGGAGCGTTACGGATAAATTCTGTCTCTACGAAGGGATCGTTTTCGGA  
 300

DpnI  
 BclI |  
 Sau3AI |  
 CjePI |  
 MnlI

HinfI  
 TfiI

BsrI  
 TspRI  
 CviJI | CviRI  
 CjePI

301  
 CTATGAAGGACTGACAAGAGAACTGATCAAGGAATCGCACTGGCTCTTGCAGAAAGTTA  
 -----  
 GATACTTCCTGACTGTTCTCTTTGACTAGTTCCTTAGCGTGACCGAGAACGTCITTCAT  
 360

DpnI  
 Sau3AI |  
 Hpy188IX  
 DraI  
 MseI | DdeI |  
 BseMII

361  
 TACCCGTGCAAAAGATCATAGGCTCTATACCTTTAAACTCGAGCCTTCGTGTTGGAGCGA  
 -----  
 ATGGGACAGTTTCTAGTATTCCAGATATGGAATTTGAGTCTGGAAGACACACCTTCGCT  
 420

ApoI  
 Tsp509I  
 HphI |  
 RsaI  
 Bst4CI | NspV |  
 TatI | TaqI |  
 BccI |  
 TspRI  
 BtsI

421  
 TGGCACTCCACTCACTGCTTATGACTTTGAAAAATCTATAAAACAACCTGTACTTCGAAGA  
 -----  
 ACCGTGAGGTGAGTGACSAATCTGAAACTTTTTAGATATTTTGTGCATGAAGCTTCT  
 480

ApoI  
 Tsp509I  
 MboII  
 MseI |  
 MboII

481  
 ATTTTCACCTTCCATACATACCTTTACTCGGCGIGATTAAAAATCTTCGGCAATCCACAA  
 -----  
 TAAAGTGGAAGGTATGATGAAATGAGCCGCACTAATTTTTAGAAAGCCGTTAGGTGTT  
 540

DpnI  
 Sau3AI |  
 Hpy178III  
 BciVI

541  
 TGCTCAAAAAATCTCTGGAACCTCTTGGGTACAGGCAAAAGATGATCTTACTTTGGTGAT  
 -----  
 ACGAGTTTTTAGAGACCTTTGAGAACCTATGTCGGTTTCTACTAGAATGAACCACTA  
 600

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Figure 11 (Cont.)

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                                     Bst132I
                                     BscGI
                                     CjePI
                                     Cac8I
                                     |
                                     |
                                     |
HphI                                     |
BfaI | CjePI                           |
|   |   |                               |
|   |   |                               |
TACCCCTAGAGCAACCTTTCCCATACTTTCTCACACTTATCGCTCGCCCGTATTCTCCCC
601 -----+-----+-----+-----+-----+-----+-----+
ATGGGATCTCGTTGGAAAGGGTATGAAAGAGTGTGAATAGCGAGCGGGGCATTAAGAGGGG
                                     660

Bs36I HinfI
DdeI   TfiI
|       |
|       |
TGTTTCATCACACCCCTTAGGGAATCCTATAAGAAAGGAACACCCCCATCCACATACATCTC
661 -----+-----+-----+-----+-----+-----+
ACAAGTAGTGTGGGAATCCCTTAGGATATCTTTCCTGTGGGGGTAGGTGTATGTAGAG
                                     720

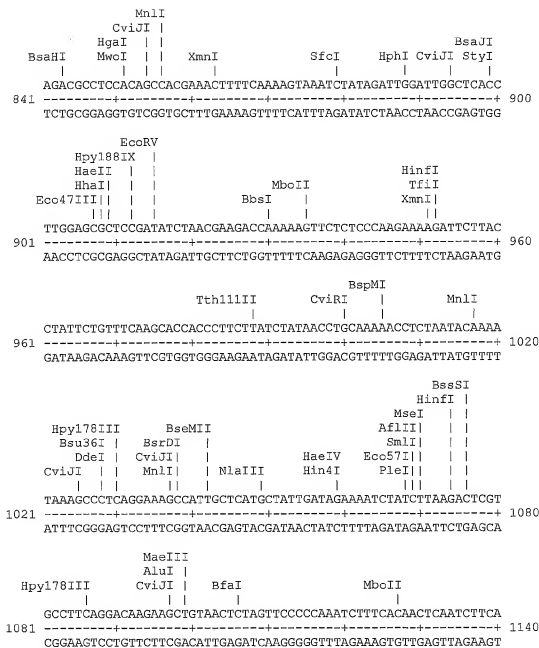
ApaI
BanII
BseSI
Bsp1286I
BmgI |
CviJI |
HaeIII |
NlaIV |
EcoO109I |
Sau96I |
Sau96I | |
| | |
MseI
NlaIII
|
|
CAATGGGCCCTTTGTCTTAAAAAACATGAACACCAAACTACTTAATTTTAGAAAAAAA
721 -----+-----+-----+-----+-----+-----+
GTTACCCGGGAACAGAAATTTTTTGTACTTGTGGTTTTGATGAATAAAATCTTTTTT
                                     780

HinfI
NlaIII
TfiI
HaeIV |
Hin4I |
Hpy178III |
RcaI | |
DpnI | | |
BclI | | | |
Sau3AI | | | |
MnlI | | | |
MslI | | | | |
| | | | |
MaeIII
Tsp45I
HinfI |
Tth111I | |
HphI | | |
| | |
TaqII
Tsp509I
MseI |
PleI |
|
TCCCTCACTACTATGATCATGAATCAGTAAAGTTAGACCGAGTCACCTTAAAAATATCCC
781 -----+-----+-----+-----+-----+-----+
AGGAGTGATGATACTAGTACTTAGTCATTTCATCTGGCTCAGTGAATTTTTTAATAGGG
                                     840

```

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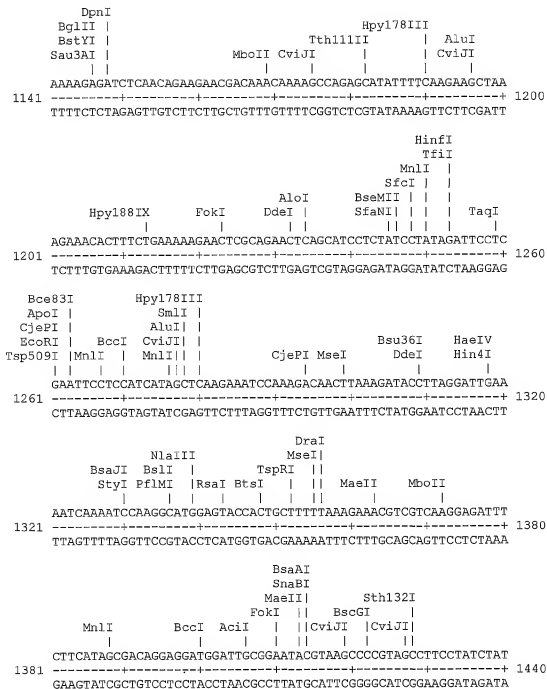
Figure 11 (Cont.)





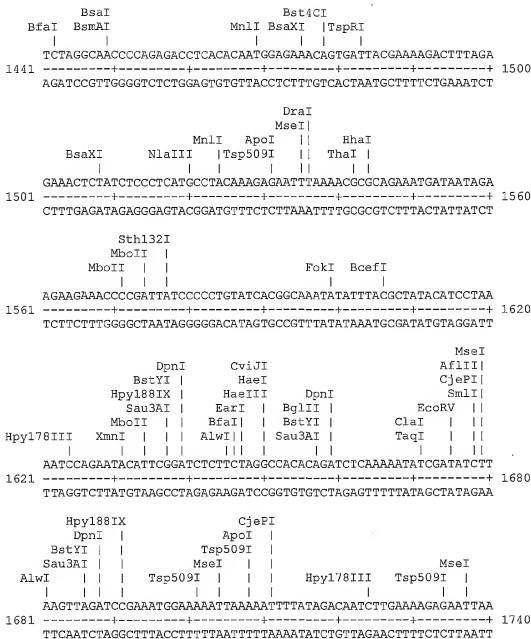
33/111

Figure 11 (Cont.)



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Figure 11 (Cont.)



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Figure 11 (Cont.)

```

                Tsp509I
          Tsp509I DraI|
    ApoI      | SwaI|      MunI
Tsp509I  MeeI|MeeI||      Tsp509I
          |  ||  |||      CviRI|      DdeI
          |  ||  |||      ||      |
AAATTTTAAATTTAAATTATAGTTGCAATTGAAAACGCCCTAAGAA
1741 -----+-----+-----+-----+----- 1787
          TTTAAAAATTAAATTTAATATCAACGTTAACTTTTGCGGGGATTCTT

```

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**Figure 12. Restriction enzyme analysis of the *C. pneumoniae* secretory locus protein gene (SEQ ID NO: 3).**



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Figure 12 (Cont.)

MunI  
Tsp509I

BbvCI | BseMII  
Bpu10I | HinfI  
DdeI | MnlI TfiI MaeIII  
Tsp45I

CGCCTCAGCAATTGAAAGAATCTCAAAGATAAAGGCCAAAGTCACTCCAAGCAGATTGC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+  
CGCGAGTCGTTAACTTCTTAGAGTTTTCTATTCCGTTTCAGTGAGGTTTCGTCTAACG

CviJI  
HaeI  
HaeIII  
StuI

AluI  
CviJI  
MwoI | DdeI Bst4CI NlaIV Bce83I  
BspMI | CviRI

GAAAGTAGCTACTAAGAAAAAGCAAAGATACC GTTTATTGCGAGTTTCCTTTTCAAGGCC  
-----+-----+-----+-----+-----+-----+-----+-----+  
CTTTCATCGATGATTCTTTTTCGTTTCTATGGCAAAATACGTCCAAGGAAAAGTTCCGG

BsmI  
SfcI

Hpy188IX SmlI DdeI Hpy178III MnlI  
MnlI | MjaIV | Sth132I

TCCGAATRACTCAAGGTATACCTCTAGCTTTGCTTAGTGAACCTCCC GAATGCATAG  
-----+-----+-----+-----+-----+-----+-----+-----+  
AGGCTTATTGAGTTCCATATTGGAGATACGAAACGAATCACTTGGAGGGCTTACGATATC

HaeIV SfaNI BtrI MjaIV  
Hin4I NlaIII MaeIII AccI  
TaqI | Sth132I

CGATACAGCATCATGGTATGCTATTTTTATTTCGGTTACTTCGACGTGCTTATSTAGACAC  
-----+-----+-----+-----+-----+-----+-----+-----+  
GCTATGTCGTAGTACCATACGATAAAAATAAGCCAATGAAGCTGCACGAATACATCTGTG

AlwI

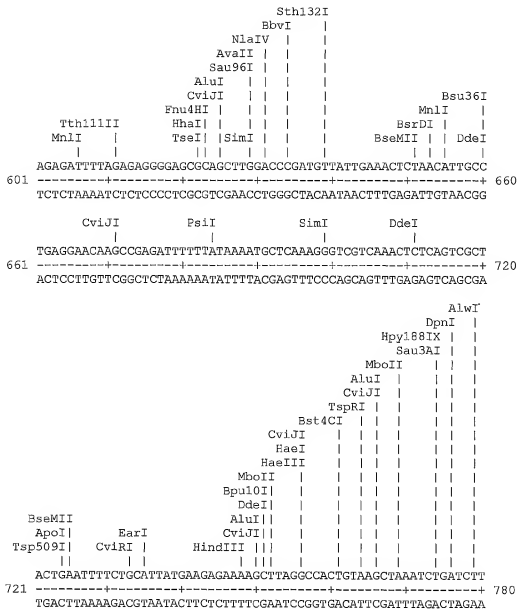
Hpy188IX  
DdeI |  
MnlI |  
DpnI |  
BstYI |  
Sau3AI |  
ScrFI |  
EcoRII |  
BseMII |

BacGI RsaI BccI MaeIII

GGGAAATGTACCTPCTGGATCTGAGTATGCCATCGCTAATGCTTTGATAAGTAACAACACA  
-----+-----+-----+-----+-----+-----+-----+-----+  
CCCTTTACATGGAGGACCTAGACTCATACGGTAGCGATTACGAAACTATTCTATTGTTGT

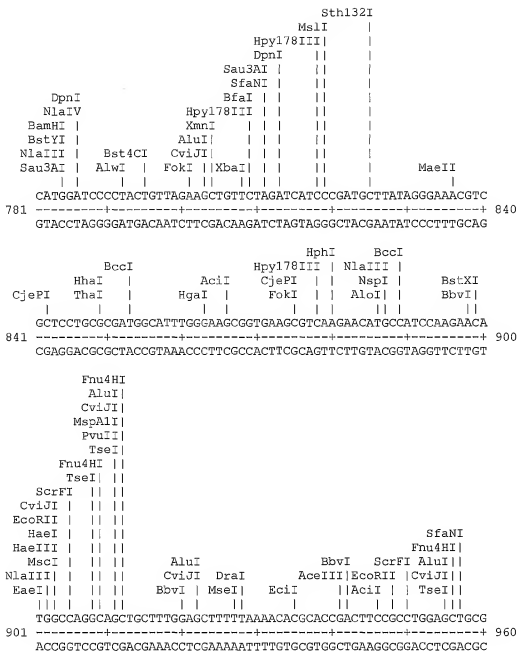
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Figure 12 (Cont.)



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Figure 12 (Cont.)



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Figure 12 (Cont.)

[illegible]



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Figure 13. Restriction enzyme analysis of the *C. pneumoniae* Endopeptidase gene (SEQ ID NO: 5).



Figure 13 (Cont.)



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Figure 13 (Cont.)

```

      CviRI
      Cac8I |
      CviJI | |
      HaeIII | |
Fnu4HI | | |
      TauI | | |
      AciI | | |      CviRI      PsiI      ApoI
      || | | |      |      |      Tsp509I      Sau3AI
      || | | |      |      |      |      |
841 AGCGGCCTGCCTACTATTGCACAAAACTTCCCACTATTATAAAGAATTTTCGTGCCG 900
      -----+-----+-----+-----+-----+-----+-----+
      TCGCCGGACGTGATGATAACGTGTTTTGAAGGGTGATAATATTTTCTTAAAGCACGGC

      AcII
      DpnI |
      DpnI |
      MseI      BstYI | |
      VspI      Sau3AI | |
      DpnI | MnlI | BsaXI | CviJI | AlwI | | |
      | | | | | | | | | | | |
901 ATCTATACTTTATGGAGGTGGCGTAGCCATTATGAATACTTTAGATCCGCAATACAAAC 960
      -----+-----+-----+-----+-----+-----+-----+
      TAGATATGAATAACCTCCACCGCATCGGTAATTACTTATGAATCTAGGCGTTATGTTTG

      BstZ17I      Hpy188IX      BseMII
      MjaIV      DdeI | Fnu4HI |
      AccI | BbvI | TseI | |
      | | | | | |
961 TCGTGTAATCTACCTGTACTTCCCCCTGCTAAACTATGCTCAGATAATGCTGCTAT 1020
      -----+-----+-----+-----+-----+-----+-----+
      ACGCACATTAGATGGACATATGAAGGGGGACGATTTGATACGAGTCTATTACGACGATA

      ApoI
      Tsp509I
      CviRI      Hpy178III |
      HaeIV      MspI |
      Hin4I      BsaWI |
      MwoI | BfaI | Tsp509I | BfaI | Kpn2I | |
      | | | | | |
1021 GATTGCAGGTCTAGGGGGGAGAAATTTTCAAAAAAACTCTAGTATTCGGGAAATTCGTAT 1080
      -----+-----+-----+-----+-----+-----+-----+
      CTAACGTCAGATCCCCCTCTTTTAAAGTTTTTTTGTAGATCATAAGGCCTTTAAGCATA

      HphI
      HinfI |
      HhaI      TfiI |
      EspI | EcoRV | TspRI | Bpu10I | MnlI |
      | | | | | |
1081 ATGCGCAAGATATCAGTGGGAATCTGTATCACCATTCTCCTTAGCCTCTCCGTAGTCTCTC 1140
      -----+-----+-----+-----+-----+-----+-----+
      TACGCGTTCTATAGTCACCCTTAGACATAGTGGTAAGAGGAATCGGAGAGGCATCAGGAG

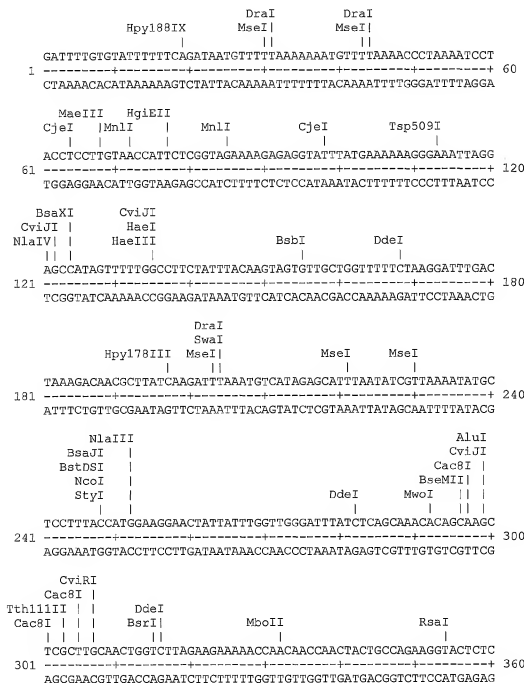
```

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Figure 13 (Cont.)

[illegible]

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Figure 14. Restriction enzyme analysis of the *C. pneumoniae* Protease gene (SEQ ID NO: 7)

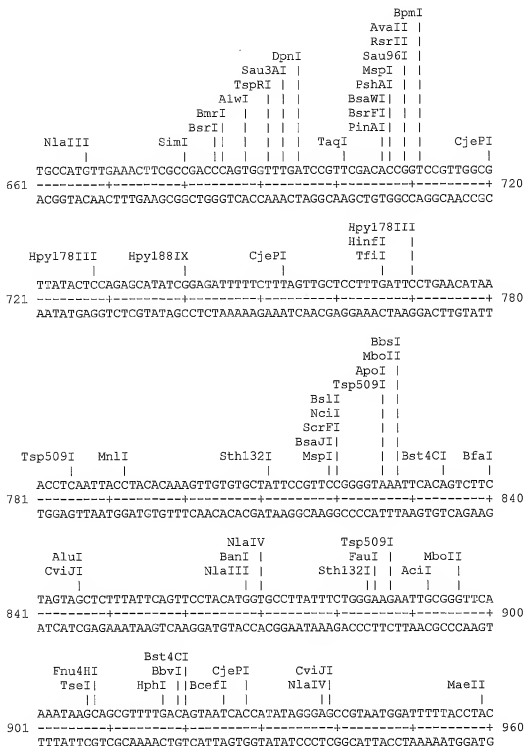
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Figure 14 (Cont.)



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Figure 14 (Cont.)

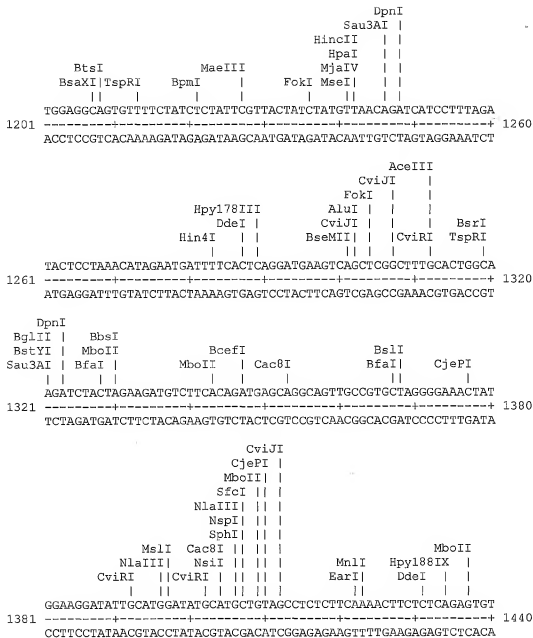






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Figure 14 (Cont.)



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Figure 14 (Cont.)

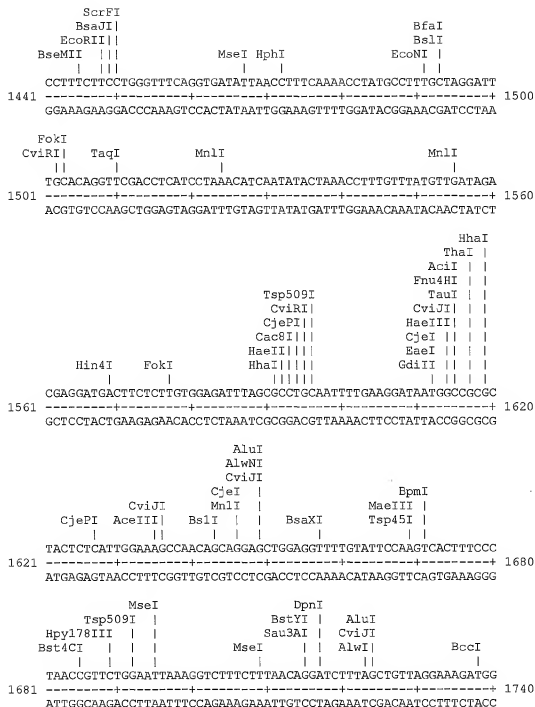
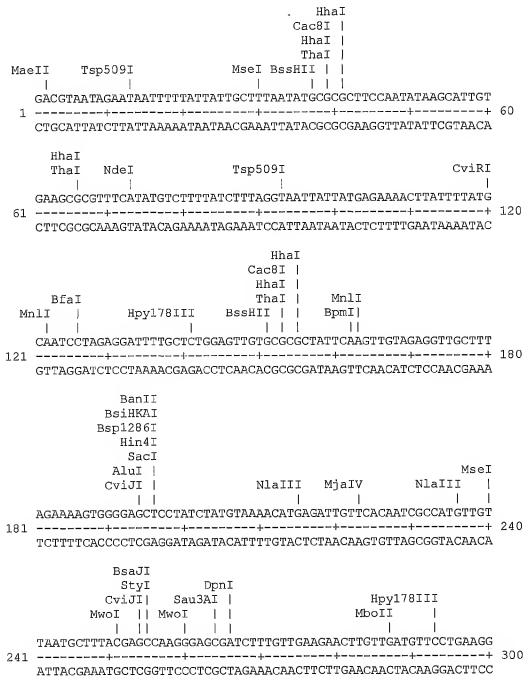


Figure 14 (Cont.)

[illegible]

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Figure 15: Restriction enzyme analysis of the *C. pneumoniae* Metalloprotease gene (SEQ ID NO: 9).

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Figure 15 (Cont.)

```

                                AceIII
                                AceIII |
                                ApoI  | |
                                EcoRI  | |
                                Tsp509I | |
                                NlaIII  | |
                                Eco57I  | | |
                                Eco57I | | |
                                PstI   | | |
                                HinfI  | | |
                                HphI   | | |
                                CviJI  | | |
                                MnlI   | | |
                                CviJI  | | |
                                CviJI  | | |
TGAGAGAGTCATTTATTCAGCTCATGGAATTCCTCCTTCAGTTAGAGCTGAAGCAAAAGC
301 -----+-----+-----+-----+-----+-----+-----+-----+
ACTCTCTCAGTAAATAAGTCGAGTACCTTAAGGAGGAAGTCAATCTCGACTTCGTTTTCG
                                SfaNI
                                Eco57I |
                                AluI  | |
                                CviJI  | |
                                Sth132I | |
                                HindIII | |
                                BscGI  | | |
                                MaeIII | | |
                                BbvI   | | |
                                BsmI   | | |
                                Fnu4HI | | |
                                TseI   | | |
                                CviJI  | | |
                                HaeIII | | |
                                EaeI   | | |
                                GdiIII | | |
                                DpnI   | | |
                                MaeII  | | |
                                MneI   | | |
                                BciVI  | | |
                                HaeIV  | | |
                                HinfI  | | |
                                Sau3AI | | |
                                MnlI   | | |
                                BsaHI  | | |
                                MaeII  | | |
                                MnlI   | | |
                                Bst4CI | | |
                                HphI   | | |
                                Hpy178III | | |
                                BcgI   | | |
                                TaqI   | | |
                                MnlI   | | |
                                BsaHI  | | |
                                MaeII  | | |
                                MnlI   | | |
GGTGATTGGTATTGTTGGAGAAGTTCCTGAACACATTACTGTTGTCGAGAAGGTTGCTGA
481 -----+-----+-----+-----+-----+-----+-----+
CCACTAACCATAACAACCTCTTCAAGGACTTGTAATGACAACAGCTCTTCCAACGACT

```

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Figure 15 (Cont.)

[illegible]





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Figure 15 (Cont.)

```

                                SfcI          FokI
                                MnlI |      Tsp509I
                                | |      CjePI |
                                | |      | |
ACAAGTGGAAAAATGATATATTTGCTGTAGAGGATGTCGTATTTC AATTACCAAAAGAACT
961 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020
TGTTCACCTTTTACTATATAAAACGACATCTCCTACAGCATAAAGTTAATGGTTTTCTTGA

                                Hpy178III
                                TaqI|
                                AvaI||
                                Hpy178III||      HinfI
                                SmlI||      MboII |
                                XhoI||      BbsI  | |
                                |||      MboII  | |
                                |||      | |
DdeI CjePI CviJI
| | |
CCGTTGTTCTTAGGTCITTAGGCTTACTTGCCAAGTTTTTCTCGAGATGCTTTATAGAG
1021 -----+-----+-----+-----+-----+-----+-----+-----+ 1080
GGCAACAAGAATCCAGAAATCCGAATGAACGGTTC AAAAGAGCTCTAACGAAATATCTC

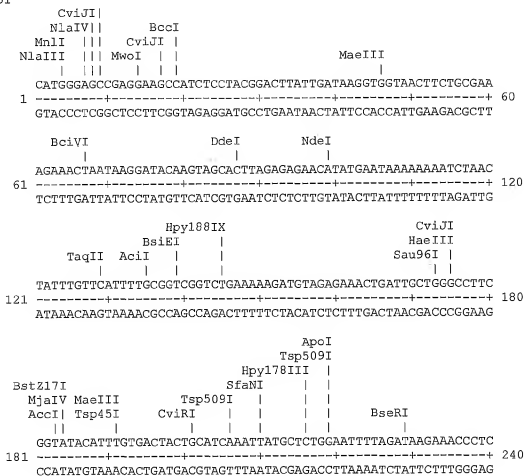
                                Hpy188IX
                                PleI MnlI |
                                | |
TCTTCTTCTCGTTCAGAGAGGGTATTTACCTTTT TTTAGTTCTCTGTATTTGAAA
1081 -----+-----+-----+-----+-----+-----+-----+-----+ 1133
AGAAGAAGAGCAAGTCTCTCCATAAATGGAAAAATCAAGAGACATAAACCTT

```

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**Figure 16: Restriction enzyme analysis of the *C. pneumoniae* CLP protease ATPase gene (SEQ ID NO: 11).**

BsaJI



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Figure 16 (Cont.)

[illegible]

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Figure 16 (Cont.)

```

Hpy178III
AlwNI|
DpnI||
BstYI |||
Sau3AI |||
SfcI | |||
CviJI | |||
HaeIII | |||
EcoO109I| |||
Sau96I| |||
BfaI || |||
AvrII| || |||
BsaJI| || |||
CjeI| || |||
StyI| || |||
BscGI
Tth111III
AluI |
CviJI |
Sth132I | MaeII
TAAACAAACAGGTAAGCTACGGGAAATCTAACGTGCTTCTCCTAGGCCCTACAGGATCTGG
421 -----+----- 480
ATTGTTGTTCATTTCGATGCCCTTTAGATTGCACGAAGAGGATCCGGGATGTCCTAGACC

Tsp509I
MseI| CjeI
AlwI VspI| CviRI
AAAAACATTAAATTGCAAAACATTGGCAAAATTTTAGATGTTCCCTTCACCATAGCCGA
481 -----+----- 540
TTTTTGTAATTAACGTTTTTGTAAACGTTTTTAAATCTACAGGGAAGTGGTATCGGCT

BspMI
HgaI |
SimI |
CGCAACGACCCCTAACGGAAGCAGGTTATGTCGGTGAAGATGTAGAGAACATTGCTTACG
541 -----+----- 600
GCGTTGCTGGGATTGCCTTCGTCCAATACAGCCACTTCTACATCTCTGTAAACAGAATGC

Fnu4HI
AluI|
CviJI|
TseI|
Sth132I
CviRI |
MnlI |
BscGI | |
Hin4I |
BsgI |
ClaI
TaqI
TTTATTACAAGCTGCTGATTACGATGTGCCCCGTGCAGAACGAGGCATTATCTATATCGA
601 -----+----- 660
AAATAATGTTTCGACGACTAATGCTACAGCGGGCACGTCTTGCTCCGTAATAGATATAGCT

```

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Figure 16 (Cont.)

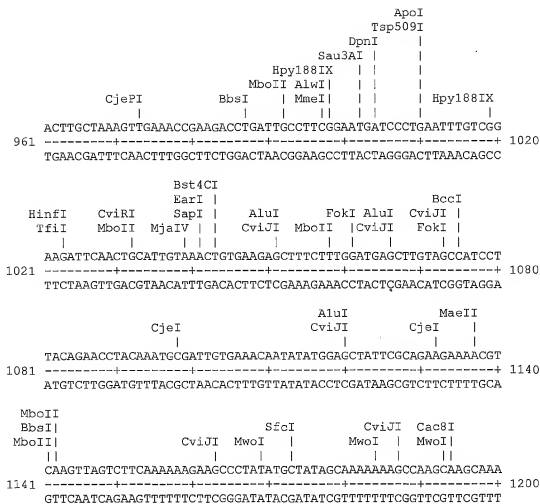
```

          Tsp509I
          ClaI   |
          TaqI   |
                                MaeII   BsmAI   BsmBI   BfaI
                                |         |         |
TGA AATCGATAAAATTGGAAGGACAACAGCAAACGTCCTCCATTACTAGAGATGTTTCTGG
661 -----+-----+-----+-----+-----+-----+-----+-----+
ACTTTAGCTATTTTAACTTCTCTGTTGTGCTTTGCAGAGGTAATGATCTCTACAAAGACC
                                Tth111III   DrdII   AclI   EcoNI
                                |         |         |         |
          XmnI   |         |         |         |         |         |
          MseI   |         |         |         |         |         |
                                |         |         |         |
CGAAGGGGTTCAACAAGCATTTGTTAAAAATCGTTGAAGGAACACAGCAACGTTCTCTCC
721 -----+-----+-----+-----+-----+-----+-----+
GCTTCCCAAGTTGTTGCTAACAAATTTTAGCAACTTCTCTGGTGTGCTTTGCAAGGAGG
                                MaeII
                                MnlII   |
                                BslII   |
EcoNI   ||   |
FokI   ||   |
BslII   ||   |         |         |         |         |
          ||   |         |         |         |         |
TAAAGGAGGACGTAAGCATCTCTAACCAAGAGTATATCCGAGTCAATACGGAAAAATATCTT
781 -----+-----+-----+-----+-----+-----+-----+
ATTTCTCTCTGCATTCGTAGGATTGGTTCTCATATAGGCTCAGTTATGCCTTTTATAGAA
                                BsaXI
                                Hin4I
                                CviJI   |         |         |         |
                                NlaIV   |         |         |         |
                                |         |         |         |
          AciI   ||   |         |         |         |         |
Pfl1108I   |   |   |         |         |         |         |
          |   |   |         |         |         |         |
ATTTATCGTAGGCGGAGCCTTCGTCAACCTAGATAAGATTATCGCAAAGCGATTGGGGAA
841 -----+-----+-----+-----+-----+-----+-----+
TAAATAGCATCCGCCCTCGGAAGCAGTTGGATCTATTCTAATAGCGTTTCGCTAACCCCTT
                                BsaI
                                DpnI   BsmAI
                                BclI   |         |         |         |
                                Sau3AI   |         |         |         |
                                Hpy188IX   |         |         |         |
                                |         |         |         |
AACTACCATAGGGTTTCTGATGATCAAGCAGACCTCTCTCAAAAAACAGAGACCATCT
901 -----+-----+-----+-----+-----+-----+-----+
TTGATGGTATCCCAAAAGACTACTAGTTGCTCTGGAGAGAGTTTTTGGTCTCTGGTAGA

```

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Figure 16 (Cont.)



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Figure 16 (Cont.)

```

                                BfaI
                                DpnI
                                BpmI
                                Sau3AI
                                AlwI
                                BfaI
                                BsiHKAII
                                Bsp1286I
                                BanII
                                BsiHKAII
                                Bsp1286I
                                SacI
                                BssSI
                                Tth111III
                                AluI
                                CviJI
                                BsrI
                                Tth111III
                                BfaI
                                DpnI
                                BpmI
                                Sau3AI
                                AlwI
                                BfaI
                                BsiHKAII
                                Bsp1286I
                                SacI
                                BssSI
                                Tth111III
                                AluI
                                CviJI
                                BsrI
                                Tth111III
                                FokI
                                BsaI
                                BsmAI
                                Eco57I
                                ApoI
                                Tsp509I
                                Bst4CI
                                SfcI
                                DpnI
                                BstYI
                                Hpy188IX
                                Sau3AI
                                AlwI
                                XmnI
                                AluI
                                CviJI
                                MboII
                                BbsI
                                CjePI
                                AATTCCTTCAGATCCTACAGTAGAAGCTATTTCATATCCAAGAAGACACTATCGCAGAAAA
                                TTAAGGAAGTCTAGGATGTCATCTTCGATAAGTATAGGTTCTTCTGTGATAGCGTCTTTT
                                NlaIV
                                AvaII
                                EcoO109I
                                Psp5II
                                Sau96I
                                Tsp509I
                                HaeII
                                HhaI
                                CjePI
                                Hpy188IX
                                SimI
                                AluI
                                CviJI
                                CjePI
                                DdeI
                                CjePI
                                TAAAGGGCCAAATAATTATCAGAAGGACCCAGAGCTATCGCTTAGCTCTTTTGTAGTTC
                                ATTTTCGGGTATTATTAATAGTCTTCTGGGGTCTTCGATAGCGAATCGAGAAAAATCAAGG

```







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Figure 17 (Cont.)

```

                                DpnI
                                BstYI |
                                Sau3AI |
                                MnlI | |
                                Hpy188IX | | |
                                AlwI | | | |
AluI      NlaIII | | | |           ApoI      ScrFI
CviJI     AceIII | | | |           MboII     Tsp509I   Tsp509I   BsaJI |
|         | | | | | | | |           |         |         |         EcoRI |
|         | | | | | | | |           |         |         |         |
GCTCCTTTTCTCATGTCCGAAGATCCTAAAAAGGATATTCAAATTTTCATCAATTCCCC
241 -----+-----+-----+-----+-----+-----+-----+ 300
CGAGGAAAAGGAGTACAGGCTTCTAGGATTTTCTCTATAAGTTTAAAAGTAGTTAAGGGG

                                BsrI
                                HaeIV |
                                Hind4I |
CviJI     MwoI | |
Fnu4HI |   BspGI | | |
HphI |     MspAII | | | |
TauI |     AclI | | | | |
AclI |     | | | | | |
| | | | | | | |
AGGCGGCTACATCACCCTGGACTGGCAATCTATGATACCATTGCTTTTTAGGTTGTGA
301 -----+-----+-----+-----+-----+-----+ 360
TCCGCCGATGTAGTGGCGACCTGACCGTTAGATACTATGGTAAGCGAAAAATCCAACAT

                                BanII
                                Bsp1286I
                                CviJI |
                                NlaIV | |
                                SfaNI | | |
                                NlaIII | | |
                                BsaJI | | | |
                                BstDSI | | | |
                                CviRI | | | | |
                                Fnu4HI | | | | |
                                AluI | | | | |
                                FokI   CviJI | | | | |
                                CviRI | TseI | | | | |
Ta qII BbvI | SfaNI | | StyI | | | | |           AclI
| | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
TGTAATAACCTACTGCATCGGTCAAGCTGCATCCATGGGAGCCCTCTTATTATCCGCAGG
361 -----+-----+-----+-----+-----+-----+ 420
ACATTTATGGATGACGTAGCCAGTTCGACGTAGGTACCCCTCGGGAGAATAATAGGCGTCC

```



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Figure 17 (Cont.)

```

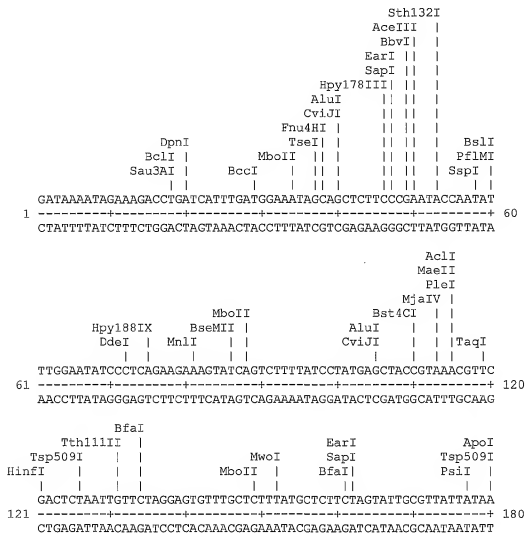
                                     Hpy188IX
                                   BsiEI
                                TaqII  AciI
                                |      |
TATGARTAAAAAACTAAGTATTGTTCATTTGCGGTGGTCTGAAGAAGATGTAGA
721-----+-----+-----+-----+-----+-----+-----+ 780
ATACTTATTTTTTTTAGATTGATAAACAGTAAAACGCCAGCCAGACTTTTCTCATCTC

                CviJI   BstZ17I
                HaeIII   MjaIV
                Sau96I | AccI|
                   ||
GAAACTGATTGCTGGGCCTTCGGTATACATTT
781-----+-----+-----+-----+-----+-----+ 812
CTTTGACTAACGACCCGGAGCCATAGTGTAAG

```

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Figure 18. Restriction enzyme analysis of the *C. pneumoniae* transglycolase/transpeptidase gene (SEQ ID NO: 15).





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Figure 18 (Cont.)

```

                                MslI
                        Hpy178III|
                        FokI  ||
                        Sth132I || ||
                        BsgI  || || ||
Hpy178III|           BsaJI           MnlI
AluI      ||      ||      ||      DpnI      |  HinfI      ||
CviJI     ||      ||      ||      |Sau3AI | StyI   TfiI      ||
|          ||      ||      ||      ||      ||      ||
TTTAGCTATTCCCGAATGTCATCGTGATGAGATCATCCAAGGGATTCTCCAAATTTATTGA
361 -----+-----+-----+-----+-----+-----+-----+
AAATCGATAAGGGCTTACAGTAGCACTACTCTAGTAGGTTCCCTAAGAGGTTAAATAACT

                                BsaXI           HaeIV           AluI
                        Pfl1108I HinfI|           MnlI           HinfI           CviJI
                        |          ||           |          ||           |
GGGGCAGACCTACGACGACCTCTCCCTAAAGTTAGATAAGAAATCTCGGTATTGTAAGCT
421 -----+-----+-----+-----+-----+-----+-----+
CCCCGTCGTGATGCTGCTGCAGAGGGATTTCATCTATTCTTTAGAGCCATAACATTCGA

                                CviJI
                                HaeIV|
                                HinfI|
                                MspI ||
                                BsrFI ||
BciVI           NlaIII|| ||           BslI           CviRI
|              ||| ||           |           |
GTATCCTTTTATTAGATGTTTCTGTCCATGACCGGTATCCCTTTGGTGGAAAGGATATGC
481 -----+-----+-----+-----+-----+-----+-----+
CATAGGAAATAATCTACAAAGACAGGTACTGGCCGATAGGGAAACCACCTTTCCTATACG

                                BaeI
                                CjeI|
                                ||
AACAAAGCATCGCTTACCAACAAACGCCCTATTTTTATTACGGACTACCAACGCTCGTA
541 -----+-----+-----+-----+-----+-----+-----+
TTGTTTCGTAGCGAATGTTGTTTTCGGGATAAAAAATAATGCCTGATGTTGCGAGCAT

                                BsaJI
                                StyI
                                BaeI |
                                CjeI |
                                AluI| |
CviJI| |           BsaXI           MseI
CjePI  BciVI || | HinfI|           SmlI|           MseI
|       |       || |           |           |           |
TCCTTTTGGGAAGCTCCTTGGACAAGTTCTCCATACCTTAAAGAGAAATTAAGGATGAGAA
601 -----+-----+-----+-----+-----+-----+-----+
AGGAAAACCCCTTCGAGGAACCTGTTCAAGAGSTATGGAATCTCTTTAATCTTACTCTT

```

Figure 18 (Cont.)

[illegible]



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Figure 18 (Cont.)

```

                                TaqI
                                BaeI |
Tsp509I PsiI RleAI           HhaI | SfaNI
    |      |      |      |      |
TCCCACAAATTTATBAGGAATACTTCAANTAACAAAGAGCGCATCGAACATACGAAGGTATC
961 -----+-----+-----+-----+-----+-----+-----+ 1020
AGGGTGTTTAATATTCCTTATGAAGTTATTGTTTCTCGCGTAGCTTGTATGCTTCCATAG

                                NlaIII
                                Hpy178III |
                                RcaI| |
                                DpnI|| |
                                Sau3AI ||| |
                                Sth132I ||| |
                                TaqI| ||| |
                                SimI || ||| |
                                NciI | || ||| |
                                ScrFI | || ||| |
                                SmaI | || ||| |
                                MspI | || ||| |
                                NciI | || ||| |
                                ScrFI | || ||| |
                                Sth132I | || ||| |
                                BaeI | BsaJI ||| ||| ||| Bst4CI
                                |      |      |      |
TTTTGTGAGCGATGTTTTTGAACCCGGGTCGATCATGAAACCTTTGACTGTGGCGATTGC
1021 -----+-----+-----+-----+-----+-----+ 1080
AAAACACTCGCTACAAAACCTTGGGCCAGCTAGTACTTTGGAAACTGACACCGCTAACG

                                MboII
                                MseI
                                AluI |
                                CviJI |
                                MnlI |
                                EarI|
                                AluI||
                                CviJI||
                                MwoI |||
                                | |||
                                Cac8I |
                                BfaI| |
                                CviJI||
                                NheI|| |
                                | |||
                                Hpy178III
                                DpnI |
                                Sau3AI | |
                                HaeIV | | |
                                Hin4I | | |
                                AlwI | | | |
                                | | | |
TTTACAAGCTAAACGAAGAGGCTAGCTTAAATCGCAGAAAAGATTTTGTATCCTGAAGA
1081 -----+-----+-----+-----+-----+-----+ 1140
AAATGTTTCGATTGCTTCTCCGATCGAATTTTAGCGTCTTTTCTAAAACTAGGACTTCT

```

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Figure 18 (Cont.)

```

                                MboII
                                ScrFI
                                Eco57I|
                                EcoRII||
                                MaeIII |||
                                Tsp45I |||
                                MboII | |||
                                ClaI | | |||
                                TagI | | |||
                                | | | |||
                                BspGI
                                ScrFI |
                                EarI |
                                BsaJI || |
                                EcoRII || |
                                | | | |||
                                MaeI
                                AflII|
                                DpnI
                                BstYI |
                                AlwI ||
                                Sau3AI |
                                AciI |||
                                | | | |||
ACCTATCGATGTGACCAGGACACTCTCCCTGGACGAAAAGGATCTCCGCTTAAGGATAT
1141 -----+-----+-----+-----+-----+-----+-----+-----+ 1200
TGGATAGCTACACTGGTCCCTGTGAGAAGGACCTGCTTTTCCTAGAGGCGAATTCCTATA

                                Hpy178III
                                MboII|
                                CviJI ||
                                NlaIII| ||
                                CjePI || ||
                                BfaI
                                RsaI | || ||
                                Hpy178III MunI BsrGI | | | | ||
                                XbaI| Tsp509I TatI | | | | ||
                                | | | | || |
                                TTCTAGAACTCTCAATTGAATATGTACATGGCTATCCAGAAATCTTCGAATGTCTATGT
1201 -----+-----+-----+-----+-----+-----+-----+-----+ 1260
AAGATCTTTGAGAGTTAACTTATACATGTACCGATAGGTCTTTAGAAGCTTACAGATACA

                                CviJI
                                Cac8I |
                                MwoI |
                                AluI || |
                                CviJI || |
                                MspAlI || |
                                PvuII || |
                                Bpu1102I || | |
                                DdeI || | |
                                AluI || | |
                                CjePI || | |BseMII
                                CviJI || | |AciI| SfaNI
                                || || | || |
                                KpnI
                                NlaIV |
                                RsaI |
                                BanI | | |
                                ScrFI | | |
                                CviJI | | |
                                EcoRII | | |
                                HaeI | | |
                                HaeIII | | |
                                CjeI | | |
                                || || | || |
AGCTCAGCTGGCTGACCGCATCATACAATCTTTAGGAGTGGCCTGGTACCAACAGAAGTT
1261 -----+-----+-----+-----+-----+-----+-----+-----+ 1320
TCGAGTCGACCGACTGGCGTAGTATGTTAGAAATCCTCACCAGCATGGTTGCTTCTCAA

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Figure 18 (Cont.)

```

                                BmrI
                                BsrI|
                                MnlI ||
                                AlwI |||
                                AluI|||
                                CviJI||| CviJI
                                TaqI ||| HaeI
                                DpnI||| HaeIII
                                MboII||| StuI
                                Sau3AI||| TspRI|
                                ||| ||| |||
GCTAGCTCTGGGATTGGGAAGAAAACAGGGATCGAGCTTCCCAGTGAGGCCCTCTGGITT
-----+-----+-----+-----+-----+-----+-----+-----+
1321 CGATCGAGACCTTAAACCTTCTTTTGTCCCTAGCTCGAAGGGTCACTCCGAGACCAA 1380

                                ScrFI
                                BsaJI |
                                EcoRII |
                                NlaIV ||
                                DrdII|||
                                BstXI |||
                                NlaIV MseI ||| AvaII
                                BanI | MslI ||| Sau96I
                                MnlI | VspI ||| BslI |
                                ||| ||| |||
GGTGCCTTCTCCCCATCGTTTCCATATTAAATGGTTCCTGGAATGGTCCTTAICTACTCC
-----+-----+-----+-----+-----+-----+-----+
1381 CCACGGAAGAGGGGTAGCAAGGTATAATTACCAAGGGACCTTACCAGGAATAGATGAGG 1440

                                CviJI SspI BclVI DrdII CviJI
                                | | | |
ATATTCTTTGGCTATGGGATATAATATTTTGGCAACAGGGATACAAATGGTTCAAGCCTA
-----+-----+-----+-----+-----+-----+-----+
1441 TATAAGAAACCGATACCCATACCTATATTATAAACCGTTGTCCCTATGTTTACCAAGTTCGGAT 1500

                                BceFI CviJI DpnI
                                CviRI| HaeIII
                                MnlI| Sau96I| Sau3AI |
                                MwoI || MspI || Eco57I ||
                                ||| ||| |||
CGCTATCCTTGCAAAACGGAGGTATGCCGTCCGGCCCACTTTAGTAAAAAGATCGTCTC
-----+-----+-----+-----+-----+-----+-----+
1501 CGCATAGGAACGTTTGCCTCCAATACGGCAGGCCGGGTGAAATCATTTTTTCTAGCAGAG 1560

```

Figure 18 (Cont.)

[illegible]

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Figure 18 (Cont.)

```

                                ApoI
                                Tsp509I
Hpy188IX |
BanII | |
BsiHKA I | |
Bsp1286I | |
SacI | |
AluI | |
CviJI | |
MnlI | | |CjeI | SfaNI | CjePI |
                                Hpy178III
                                DpnI |
                                Sau3AI | |
                                CjeI | | |
                                AlwI | | |
                                BsmAI | | |
                                BsmBI | | | BslI |
GAGCTCGGAGGGAAATTTCCACCTTTAGTGATGCTCGTCTCCATAGATGATCCTGAATA
1801 -----+-----+-----+-----+-----+ 1860
CTCGAGCCTCCCTTTAAAGGGTGGAAATCACTACGAGCAGAGGTATCTACTAGGACTTAT

                                NlaIV
                                BanI |
                                Fnu4HI | |
                                TauI | |
                                AciiI | |
CviJI |
Cac8I |
CjePI | | Tsp509I BceFI
TGGTTTTCGAGCCGACGGCAGCAAAAAATTATATGGGGGGCGTGTGCGGCACCCATT
1861 -----+-----+-----+-----+-----+ 1920
ACCAAACGCTCGGCTGCCGTGCTTTTAAATATACCCCCCGCAACACGCGTGGGTAAAA

                                Hpy178III
                                HinfI |
                                TfiI | |
                                DdeI | |
                                BcgI | |
TaqII | AciI | MnlI | | AluI | |
BfaI | BseRI | MboII | | CviJI | |
TTCTAGGGTTGCTGACCGCACACTCCTCTATTTAGGGATTCTTCCAGACAAGAAGCTAAG
1921 -----+-----+-----+-----+-----+ 1980
AAGATCCCAACGACTGGCGTGTGAGGAGATAAATCCCTAAGAAGGTCTGTTCCTCGATTTC

                                MseI
                                CviRI |
                                MboII |
                                Fnu4HI | |
                                HgaI | |
                                TseI | |
                                Fnu4HI | | |
                                BbvI | | |
                                BbvI | | | CviJI | | |
Tsp509I | | | TseI | | | BcgI | | |
                                BsmAI |
                                BsmBI |
                                HinfI |
                                TfiI |
                                Hpy188IX
                                MboII |
AAATTGCGACGAAGAAGCTGCTGCATTAAAGCGTCTCTATGAAGAATGGAATCGTCTCC
1981 -----+-----+-----+-----+-----+ 2040
TTTAACGCTGCTTCTTCGACGACGTAATTTTCGAGAGATACTTCTTACCCTAGCAGAGG

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Figure 18 (Cont.)

```

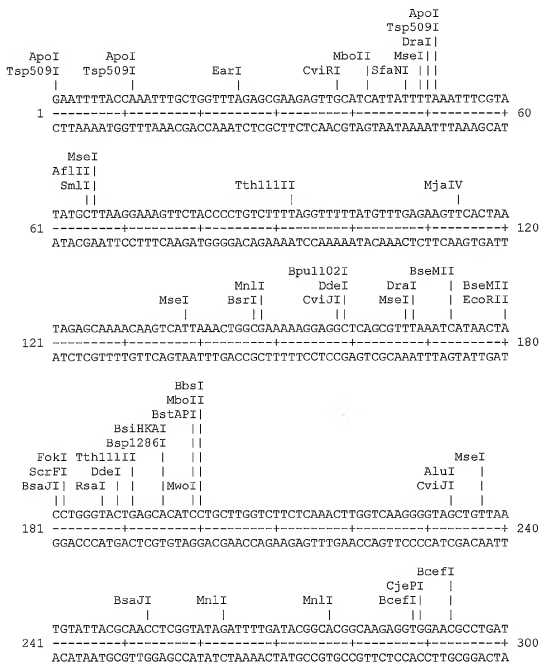
                                DpnI
                        MnlI   BstVI | AlwI
        BslI   | MnlI   Sau3AI | HphI| BccI   SfcI           Bst4CI
        |      |      |      |      |      |      |
2041  GAAACARGGGGGACGAGGTGAGGATCTCTATTTCCATCTTGCTATAGACTTTTACCGTT  2100
-----+-----+-----+-----+-----+-----+-----+
        CTTTGTTC CCCCTTGCTCCACTCCTAGAGATAAAGGTAGAACGATATCTGAAAATGGCAA

                                BsmAI
                                BsmBI
                                Sth132I|
                                BanII   ||
                                BscGI   ||
                                Bsp1286I ||
                                Hin4I   ||
                                CviJI   ||
                                Hin4I   ||
                                BseRI   ||
                                Hpy188IX ||
                                HaeIV   ||
                                Hin4I   ||
        PleI  HinFI   |      |      |      |      |      |      |      |      |
        |      |      |      |      |      |      |      |      |
2101  GAGCAAAGACTCTCTATCAGAGAGCCCGTCTCCTCTTTATCCTCTATGAGTAGTTTATGT  2160
-----+-----+-----+-----+-----+-----+-----+
        CTCGTTTCTGAGAGATAGTCTCTCGGGCAGAGGAGAAATAGGAGATACTCATCAATACA

        TA
2161  -- 2162
        AT

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Figure 19. Restriction enzyme analysis of the *C. pneumoniae* CLPc protease gene (SEQ ID NO: 17).

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Figure 19 (Cont.)

```

              ApoI
            Tsp509I      MjaIV
              BspGI |    AccI|
Hpy178III |    |    BsaI|
AvaII |    |    BsmAI|
Sau96I |    |    CjePI ||    SimI      EarI      MboII
      |    |    |    |    |    |    |
301 TGGTTATGGTCCAGAAATTCAGTCTACGGAGACCOCTGCCCTTACAGGAAGAGTAAAAAA
-----+-----+-----+-----+-----+ 360
ACCAATACCAGGTCCTTTAAGTTCAGATGCCTCTGGGACGGGAATGTCCTTCTCATTTTTT

              MboII      Hpy178III
              CviJI |    MslI
              Cac8I |    |
              CviJI | |    Tsp509I
HinfI |    MnlI |    |    HaeI | |    BsiHKAI| |
TfiI |    EarI|    |    HaeIII | |    Bsp1286I| |
      |    |    |    |    |    |
421 ATCTTTTGAATCAGCAAAATGAAGAGGCCAGCCTTTTAGAGCACAAATATGTGGGACGGA
-----+-----+-----+-----+ 480
TAGAAACTTAGTCGTTACTTCTCCGGTCGGAAAATCTCGTGTTAATACAGCCCTGCC'

              AlwI
              HaeIV |
              Hin4I |
              DpnI | |
              NlaIV | |
              BamHI | | |
              BstYI | | |
              Sau3AI | | |
              AlwI | | |    MboII
              DdeI | | |    |    EarI
BsmFI | | |    |    |    |    SapI
      | | |    |    |    |
421 GCATTACTCTTAGGGATCCTACATCAATCAGATAGTGTGCTCTTCAGGTATTAGAAAA
-----+-----+-----+-----+ 480
CGTAAATGAGAATCCCTAGGATGTAGTTAGTCTATCACAGCGAGAAGTCCATAATCTTTT

              MnlI
              DpnI|
              Sau3AI ||
              ClaI ||
              TaqI ||
              AlwI || |
              CjeI || |    ApoI CjeI |    HaeIV
              Tsp509I DdeI| |    |    Hin4I
              | | |    |    |    |
481 CTTACATATCGATCCAAGAGAGGTCGTAAGGAAATTCCTTAGAGAATTAGAGACCTTCAA
-----+-----+-----+-----+ 540
GAATGTATAGCTAGGTTCTCTCCAAGCATTCCTTTAAGAAATCTCTTAATCTCTGGAAGTT

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Figure 19 (Cont.)

```

          FokI
        MboII |
          MnlI |
          BbsI ||
        MboII ||
          | ||
TCTACAACCTTCCTCCTTCGTCGTCCTTCTTCCTCATCCTCTCGAAGCAACCCCTTCATC
541 -----+-----+-----+-----+-----+-----+-----+ 600
AGATGTTGAAGGAGGAAGCAGCAGCAGAAGAAGGAGTAGGAGAGCTTCGTTGGGAAGTAG

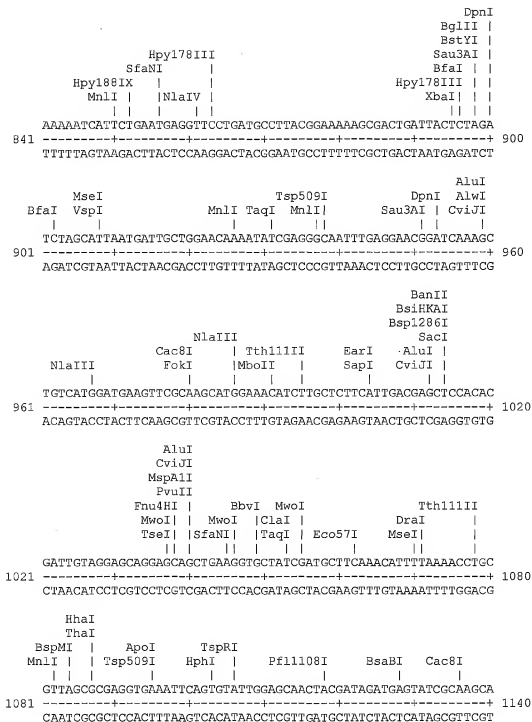
          Bpu10I
          DdeI
          AluI |
          CviJI | Hpy188IX
          || Hpy188IX
          ||
TTCAAAATCTCCTTTAGGTCATAGCTTAGGTTCTGACAAAAACGAAAAGCTTTCTGCTCT
601 -----+-----+-----+-----+-----+-----+-----+ 660
AAGTTTTAGAGGAAATCCAGTATCGAATCCAGACTGTTTTTGCTTTTCGAAAGACGAGA

                                          Eco57I
                                          DpnI |
                                          Sau3AI | |
                                          TaqI | | |
          HinfI
          Hpy188IX | AluI | | |
          AvaII | | CviJI | | |
          Sau96I | | PleI | | |
          CjePI | | AlwI | | | MboII
MwoI NdeI | MseI | BccI | | DdeI | | | | BslI |
          | | | | |
GAAAGCATATGGTTATGATTTAACGGAGATGGTCCGAGAGTCTAAGCTCGATCCTGTCTAT
661 -----+-----+-----+-----+-----+-----+-----+ 720
CTTTCGTATACCAATACTAAATTCGCTCTACCAGGCTCTCAGATTCGAGCTAGGACAGTA

          Bst4CI
          Hpy188IX TaqI | HinfI
          | | TfiI
          | |
TGGTCGTTCTTTCAGAAGTCGAACGGTTGATTTTGATTCCTTGCCGAAGAGAAAAACAA
721 -----+-----+-----+-----+-----+-----+-----+ 780
ACCAGCAAGAAGTCTTCAGCTTGCCAACTAAAACTAAGAAACGGCTTCTTCTTTTGTGTT

                                          BpmI
                                          MnlI |
                                          MunI |
          RsaI
          TatI | AluI
          | | Tsp509I | |
          CviJI | | CviRI | | SimI CviJI
          | | | |
TCCGTGTACTTATTGGAGAAGCTGGAGTTGGTAAGACTGCAATTGTTAGGGTCTGGGCTCA
781 -----+-----+-----+-----+-----+-----+-----+ 840
AGGACATGAATAACCTCTTCGACCTCAACCATTCTGACGTTAACAACTCCCAGACCGAGT

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Figure 19 (Cont.)

```

          BsaXI          DpnI          BanII
          Hin4I|          BglII|          Bsp1286I
          MaeII ||          BstYI|          AluI  BssSI|
          NlaIII ||          MnlI|          Sau3AI|          CviJI CviJI||
          || ||          ||          ||          ||
1321  TGGACGTTTCCTCCCTGATAAAGCAATAGATCTTTTAGATGAAGCTGGGGCTCGTGTCCG
-----+-----+-----+-----+-----+-----+-----+-----+
          ACCTGCAAGGAGGGACTATTTTCGTTATCTAGAAAATCTACTTCGACCCCGAGCACAGGC
1380

          SfcI          BfaI
          CviJI|          AluI|
          SimI ||          CviJI|
          || ||          MseI MnlI || CviJI TaqI
          || ||          || || ||
1381  TGTGAATACAATGGGTCAGCCTACAGATTAAATGAAGCTAGAGGCTGAAATCGAAAATAC
-----+-----+-----+-----+-----+-----+-----+
          ACACITATGTTACCCAGTCGGATGTCTAAATTACTTCGATCTCCGACTTTAGCTTTTATG
1440

          EarI
          BsaAI|
          MaeII||
          MjaIV |||
          PstI |||
          CviRI |||
          Fnu4HI |||
          CviJI CviJI          SfcI |||
          HaeI HaeI          AluI |||
          HaeIII HaeIII          AluI |||
          MscI Cac8I |          BbvI          CviJI|||
          EaeI Bce83I ||          Hpy178III |          TseI|||
          Tsp509I | CjePI |||          SmlI |||          CjePI|||
          || |||          BcgI |||          BspMI |||
          || |||          || |||
          AAAATTGGCCAAAGAGCAGGCCATTGGAAGCTCAGAATACGAAAAGCTGCAGGTTTACG
1441  TTTTAAACGGTTTCTCGTCCGGTAACCTTGAGTTCTTATGCTTTTTTCGACGTCCAAATGC
-----+-----+-----+-----+-----+-----+-----+
          MaeII
          NruI |
          ThaI |
          Hpy178III|
          BcgI MboII || CviRI          EarI
          || ||          || SapI
          || ||          ||
1501  TGATGAAGAGAAAAAAGCTTCGCGAACGTCTGCAAGATATGAACAGGAATGGGAAAATCA
-----+-----+-----+-----+-----+-----+-----+
          ACTACTTCTCTTTTTTGAAGCGCTTGAGACGTTTCATACTTTGTCTTACCTTTTATG
1560

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Figure 19 (Cont.)



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Figure 19 (Cont.)

```

      MspI
      BsaBI
      BsrFI
      PnaAI
      CviJI
      HaeIII
      Sau96I
BsaJI
  StyI
      BslI
      CviJI
      Cac8I
      Tsp509I
      SfcI
      CjePI
1801 ----- 1860
      GGAACCCGGATGGCCACATCCCTTTTCGGACGAGCGGGTTGTTTAAACGATATCTCTACAA

      ApoI
      Tsp509I
      NlaIII
      BbvI
      RsaI
      HgaI
      MboII
      CjePI
      HinfI
      TfiII
      Hpy188IX
      HphII
      BbsI
      AflIII
      PciI
      MjaIV
      AccI
      NspI
      Fnu4HI
      TseI
1861 ----- 1920
      CGSTGGTGAAGACGCTCTGATTACAGTAGACATGTCAGAGTACATGGAGAAATTCGCTGC
      GCCACCACTTCTCGAGACTAGTCCATCTGTACAGTCTCATGTACCTCTTTAAACGAGC

      DpnI
      CjeI
      Sau3AI
      BccI
      BstXI
      HphII
      BpmI
      ScrFI
      AlwI
      EcoRII
      NlaIII
      Hpy178I
      MnlI
      RcaI
      MboII
      CviJI
      HaeIII
      NlaIV
      Sau96I
      BslI
1921 ----- 1980
      TACCAAGATGATGGGATCACCTCCAGGATATGTAGGTCTATGAAGAAGGGGCCACCTTAC
      ATGTTCTTACTACCTTACTGGAGGTCTTATACATCCAGTACTTCTCCCCGGTGAATG

      MaeII
      BceFI
      RsaI
1981 ----- 2040
      GGAACAGTAGCTCGTCCGCTCCTTACTGCGTTGTTCTTCTTTGATGAGATGAAAAAGCCACA
      CCTTGTCCATGCAGCGCGAGGAATGACGCAACAAGAGAACTACTCTATCTTTTCCGTGT

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Figure 19 (Cont.)

```

                TaqII      ApoI      AatII
                AvaII |    Tsp509I    BsaHI | HinfI
                Sau96I |    CviRI      MaeII | TfiI
                | |      | |      | |
2041 CCCAGACATTATGGACCTGATGTTGCAAATTTAGAGCAAGGACGTCCTTACTGATTCTTT 2100
-----+-----+-----+
                DpnI
                Tsp509I    Hin4I |
                AceIII |    Sau3AI |
                NlaIII | |    AluI | |
                Hpy178III | |    CviJI | |
                NlaIII    RcaI | |    MnlI | |
                | |      | |      | |
2101 TGGTCGCAAAAGTGGATTTCGCTCATGCCATTATTATCATGACCTCCAATTTGGGAGCTGA 2160
-----+-----+-----+
                BsmFI
                Tsp509I |
                BplI | |
                AclI | | | CviJI      FokI      DrdI
                | | | | |
2161 TCTCATTCGTAAAAGCGGAGAAATTGGTTTGGCTTGAAGTCCCATATGGACTATAAGGT
-----+-----+-----+
                AGAGTAAGCATTTTCGCCTCTTTAACC AAAACGAACTCAGGGTATACCTGATATCCCA
                BsmFI
                Tsp509I |
                BplI | |
                AclI | | | CviJI      FokI      DrdI
                | | | | |
2220
-----+-----+-----+
                DdeI
                CviJI |
                MboII | |
                MseI | | | Bst4CI
                NlaIII | | | MseI | |
                TaqI | NspI | BseMII | | |
                | | | | |
2221 CATCCAAGAGAAAAATCGAACATGCTATGAAGAAACACTTAAAGCCTGAGTTCATTAACCG
-----+-----+-----+
                GTAGGTTCTCTTTAGCTTGTACGATACTTCTTTGTGAATTCGGAGCTCAAGTAATTGGC
                Hpy178III      FokI
                TaqI | HinfI |
                HaeIV    AvaI | MnlI | DpnI
                Hin4I    SmlI | TfiI | Sau3AI |
                BsmFI    FokI | XhoI | Hin4I | Hpy188IX |
                | | | | |
2281 TTTGGATGAAAGTGTGATTTTCCGTCGCCCTCGAGAAAGAATCTCTATCGGAGATCATCCCA
-----+-----+-----+
                APACCTACTTTCACACTAAAAGGCAGGGGAGCTCTTTCCTAGAGATACCTCTAGTAGGT

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Figure 19 (Cont.)

```

                                Hpy178III
                                TaqI|
                                AvaI||
                                SmlI||
                                XhoI||
                                BsrI|||
                                HinfI|||
                                BsmAI|||
                                DpnI      BspGI|||
                                Sau3AI|  P1eI|  |||
                                |  |  |  |||
                                TTTAGAGATCAACAACTGGACTCGAGACTGAAAACTACCAATGGCTTTGAACATCCC
2341 -----+-----+-----+-----+-----+-----+-----+-----+-----+
                                AAATCTCTAGTTGTTGACCTGAGCTCTGACTTTTGTGATGGTTTACCGAAACTTGTAGGG

                                BtrI
                                BsiHKAI|
                                Bsp1286I|
                                MaeII|
                                BpmI      CjeI
                                MaeIII|
                                AlwNI      MaeI|  |
                                HinfI|  |  BfaI|  |  Hpy178III      BceI|  |
                                |  |  |  |  BsmI|  |  BslI|  |
                                |  |  |  |  |  |  |  |
                                AGACTCTGTGATTTCTCTAGTAACGAAGGGGCATTCTCCAGAAATGGGAGCAGCTCC
2401 -----+-----+-----+-----+-----+-----+-----+-----+
                                TCTGAGACACTAAAGGAAGGATCATGTCTTCCCGTAAGAGGTCCTTTACCTCGTGAGG

                                MseI
                                BceI|  |
                                BanII|  |
                                BsiHKAI|  |
                                Bsp1286I|  |
                                SacI|  |
                                DpnI      AluI|  |
                                BstYI|  |  CviJI|  |
                                Sau3AI|  |  AcI|  |
                                AlwI      MboII|  |
                                MnlI      RsaI|  |  BfaI|  |  MnlI|  |  HinfI|  |
                                |  |  |  |  |  |  |  |
                                TCTACGCCGTGTCTATTGAGCAGTACCTTGAAGATCCTCTAGCGGAGCTCTTGCTTAAAGA
2461 -----+-----+-----+-----+-----+-----+-----+-----+
                                AGATCGCGCACAGTAACCTCGTCATGGAACCTTCTAGGAGATCGCCTCGAGAACGAATTTCT

```



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Figure 19 (Cont.)

```

                                Pfl1108I
                                AluI  |
                                CviJI |
                                Cac8I | |
                                CjePI | | |
                                Cac8I | | | |
                                Hpy178III AluI | | | | BsaJI CjePI
                                PleI  | CviJI | | | | StyI  ThaI  |
                                | | | | | | | | | |
GTCCTGCCGTCAAGAAGCTCGCAAGCTACGAGCAACCTTGGTTGAAAATCGCGTTGCCTT
2521 -----+-----+-----+-----+-----+-----+-----+----- 2580
CAGGACGGCAGTTCTTCGAGCGTTTCGATGCTCGTTGGAAACCACTTTTAGCGCAACGGAA

                                BseRI
                                Fnu4HI |
                                AluI  |
                                CviJI | |
                                TseI  | |
                                MnlI   | | | | MnlI
                                EarI  | | | | HinfI|
                                BslI  || | | | TfiI|
                                EcoNI | | BbvI MboII | | | BfaI  | BslI  ||
                                | | | | | | | | | |
TGAAAGCGAAGAAGAGGAGCAGGAAGCTGCTCTCCCTAGCCCTCACTTGGAAATCATAGGA
2581 -----+-----+-----+-----+-----+-----+-----+----- 2640
ACTTTCCTTCTTCTCTCTCGTCCTTCGACGAGAGGGATCGGGAGTGAACCTTAGTATCCT

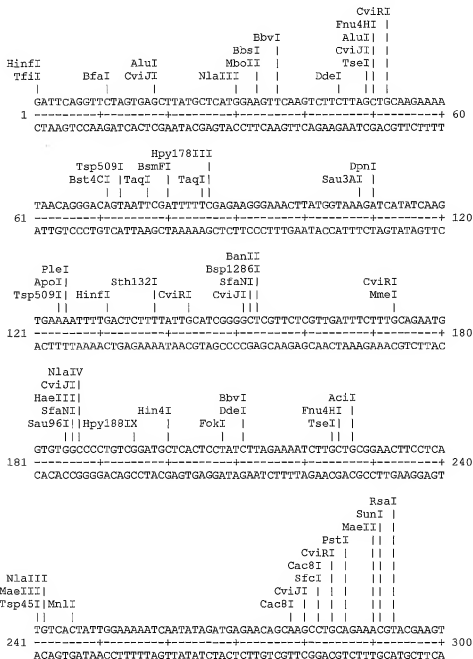
                                HgiEII
                                BsaJI  |
                                TaqI   | | | | CjeI   ScrFI
                                MaeII  | CjeI  |StyI  | Tth111III | EcoRII |
                                | | | | | | | | | |
ACGTCGATAACTCCACTACCAAGGCAGGTATCTCCTTGATAAACGCTATTGTTTGTCTCT
2641 -----+-----+-----+-----+-----+-----+-----+----- 2700
TGCAGCTATTGAGGTGATGGTTCCGTCATAGAGGAACATTTTGCGATACAAACAGGA

                                AciI
                                Sth132I BpmI
                                MaeIII  | BscGI |
                                | | | | |
GGAGTTACCGCCTTGACGGGTTGTGAAAATCGCACCTT
2701 -----+-----+-----+-----+-----+-----+-----+----- 2738
CCTCAATGGCGGAAC TGCCCAACACTTTTAGCGTGGAA

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**Figure 20. Restriction enzyme analysis of the *C. pneumoniae* Thioredoxin gene (SEQ ID NO: 19).**



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Figure 20 (Cont.)

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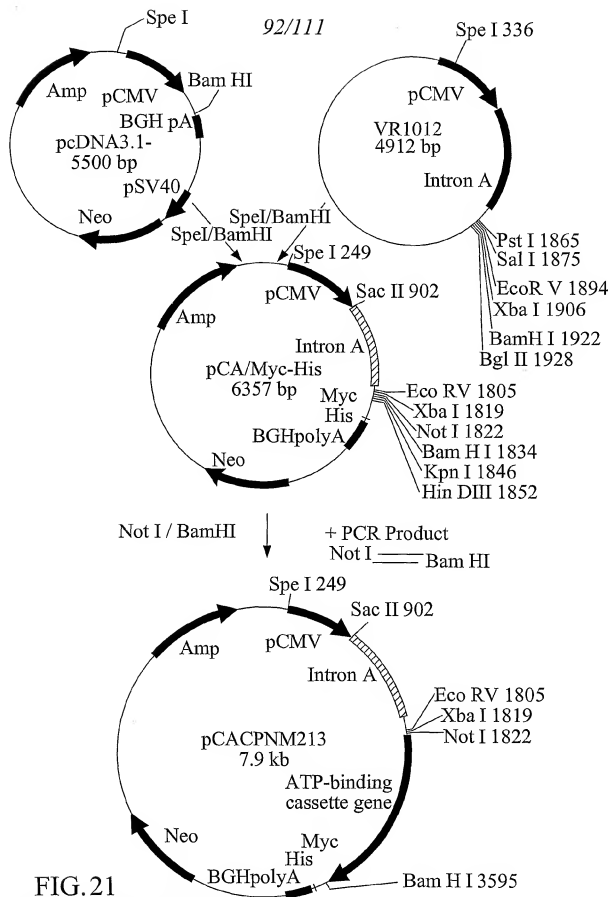
                                     AvaI
                                     CviJI|
                                     FokI  ||
                                     Sth132I  ||
                                     BccI      |||Pf11108I
                                     MnlI      |||SimI  |
                                     MseI      |||
AluI      AceIII      MseI      BccI      MnlI      |||
CviJI      |      |      |      |      |      |
|      |      |      |      |      |      |
CAGCTCTATTCTCTACGCTTATTCTTTTAAAGGATGGGAACGAGGTGGCTCGGGTCTAGG
301 -----+-----+-----+-----+-----+-----+ 360
GTCGAGATAAGGATGCGAATAAGAAAAATTCCTACCCCTTGCTCCACCGAGCCCAGCATCC

MseI      ApoI      MseI      CviRI
AflIII|      EcoRI      BbvI  |      Pnu4HI |
SmlI|      Tsp509I      Cac8I|  |      TseI  |
||      |      ||      ||      ||
TCTTAAGGATAAAGAAATTCCTAACCAATCTTATCAATAAGCAGCTTAAAAAGACGCTGC
361 -----+-----+-----+-----+-----+ 420
AGAATTCCTATTTCTTAAAGGATTGGTTAGAATAGTTATTCGTGCGAATTTTTCTGCGACG

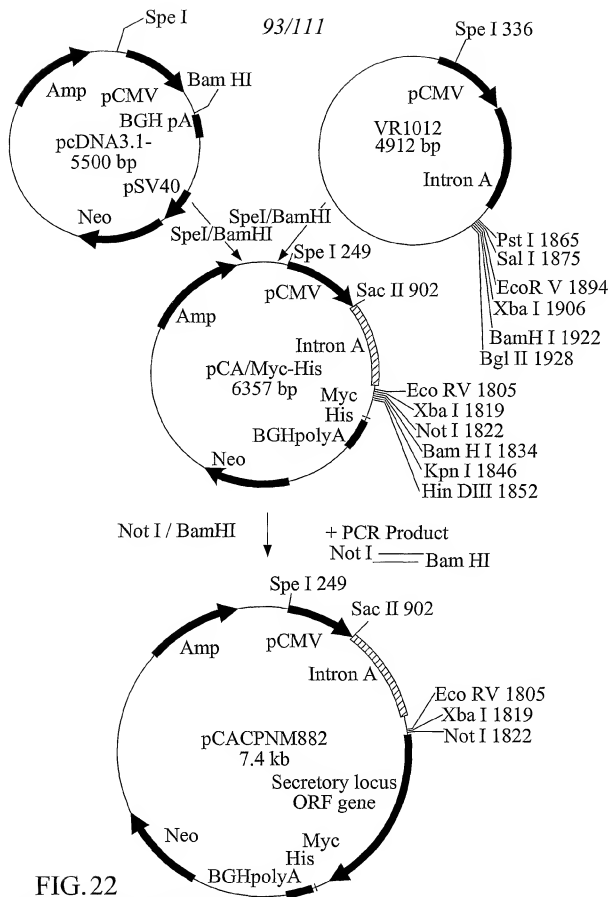
MseI
SspI  |      HinfI      Bst4CI
HgaI  |Bst4CI  TfiI      CviRI  BsrDI  |      AlwI  |
||      |      |      |      |      |
AATATTAAACCGTAGGATTCCTTTGCAATGCTACGGTTTCTGCCTTACCACCTTCATATA
421 -----+-----+-----+-----+-----+ 480
TTATAATTTGGCATCCTAAGAAAAAGTTACGATGCCAAAAGACGGAATGGTGAAGTATAT

ApoI
Tsp509I
AluI  |
CviJI |
BsrI  |
DpnI  |      TspRI  |
Sau3AI|      BslI   |      |
||      |      |      |
AAACGATCCCTACACTGGTAGCTAAATTT
481 -----+-----+-----+ 509
TTTGCTAGGGATGTGACCATCGATTTAAA

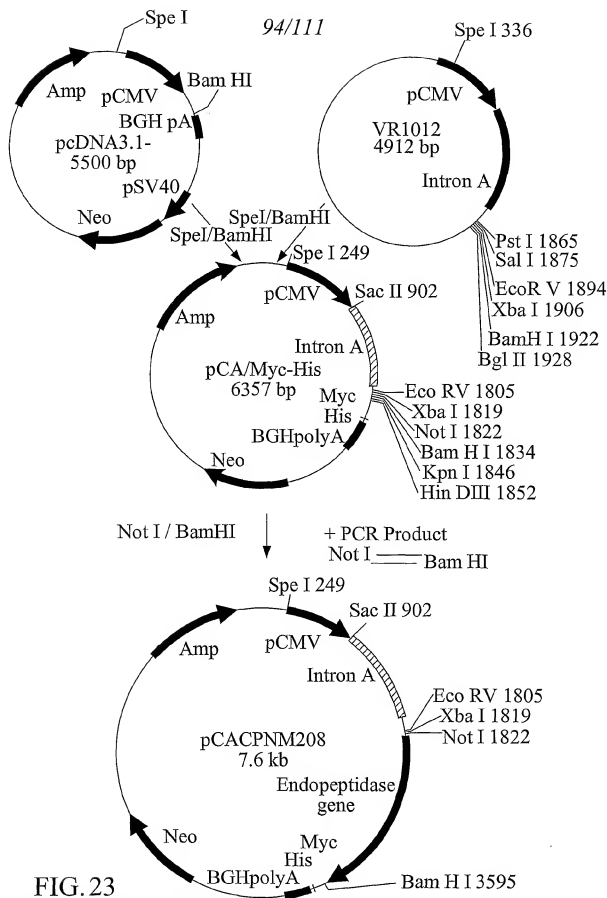
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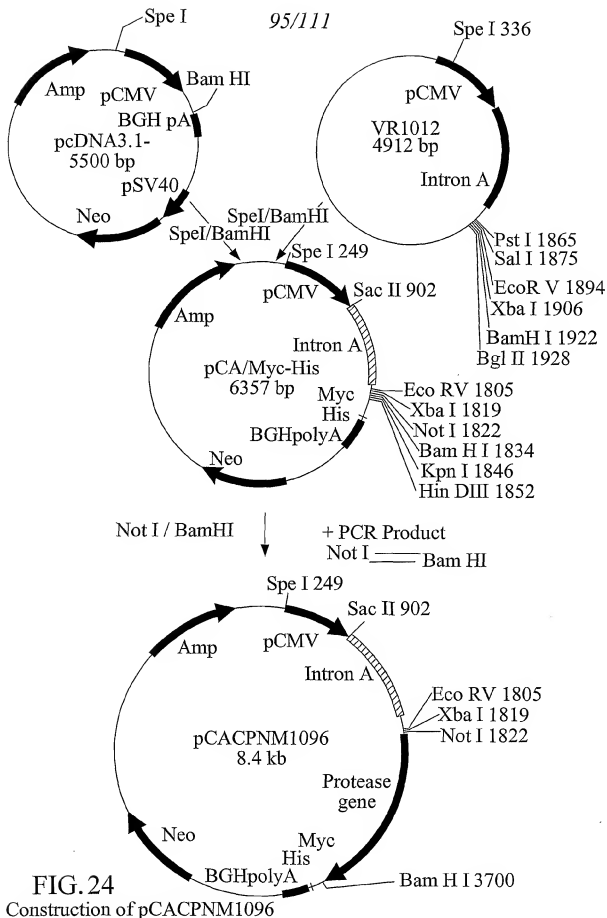
**FIG. 21**  
Construction of pCACPNM213



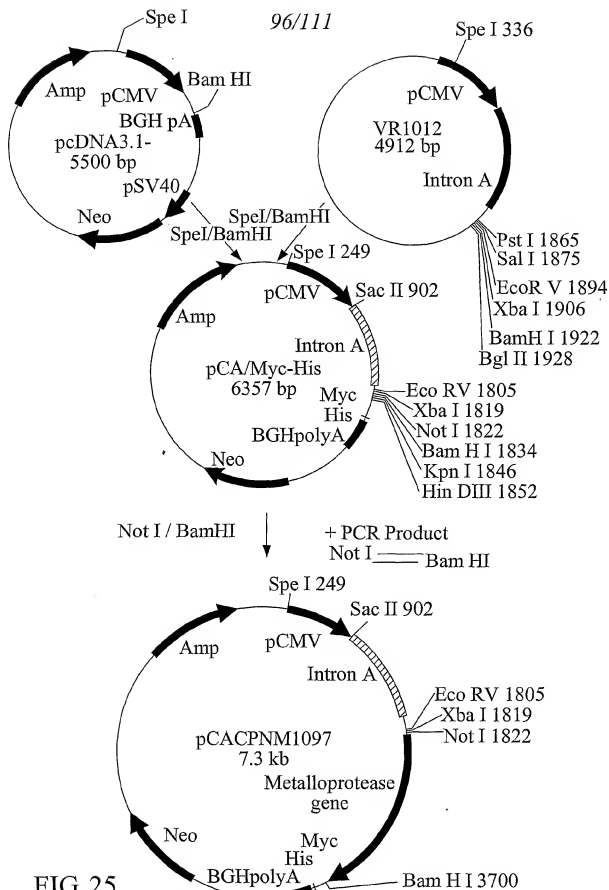
**FIG. 22**  
Construction of pCACPMM882



**FIG. 23**  
Construction of pCACPNM208

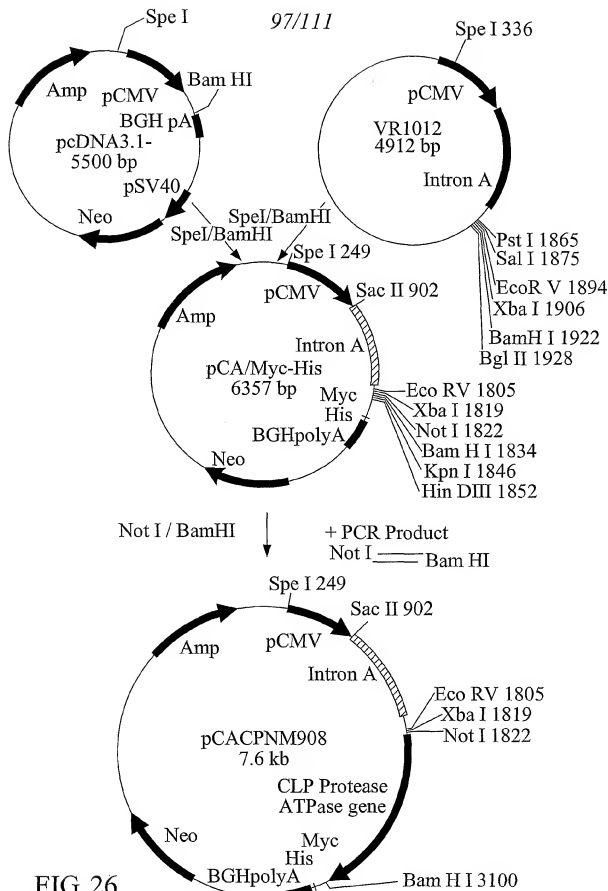


**FIG. 24**  
Construction of pCACP NM1096

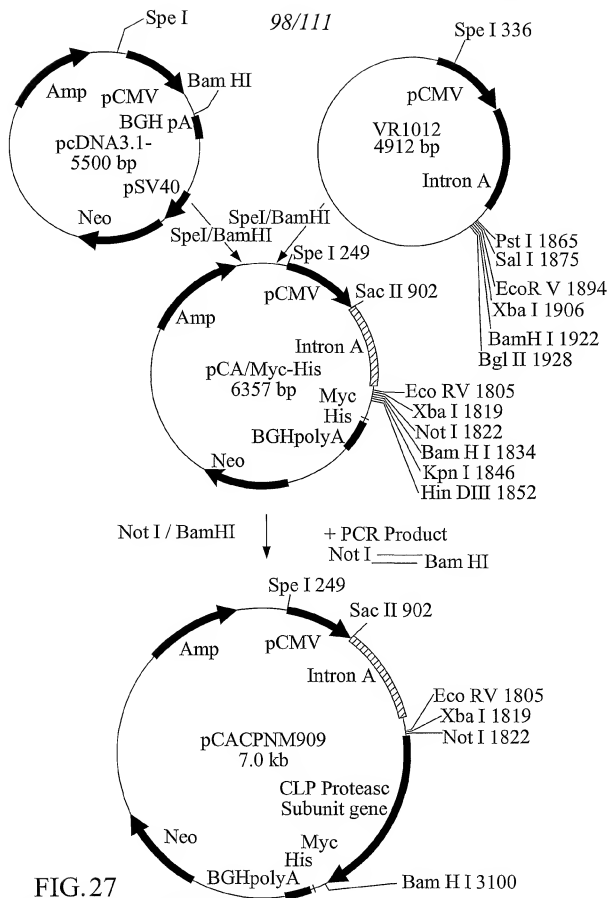


**FIG. 25**  
Construction of pCACPNI1097

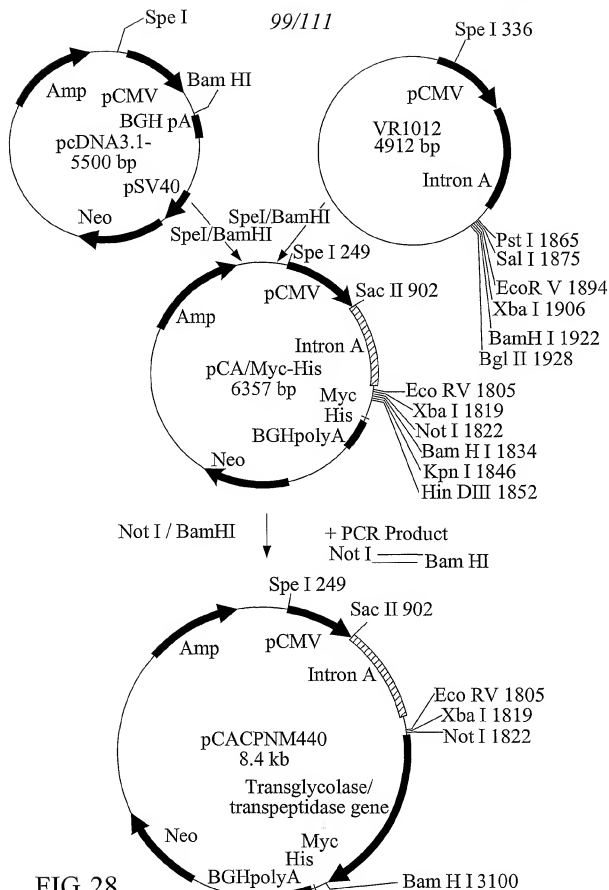




**FIG. 26**  
Construction of pCACP NM908



**FIG. 27**  
Construction of pCACPNM909



**FIG. 28**  
Construction of pCACPNM440

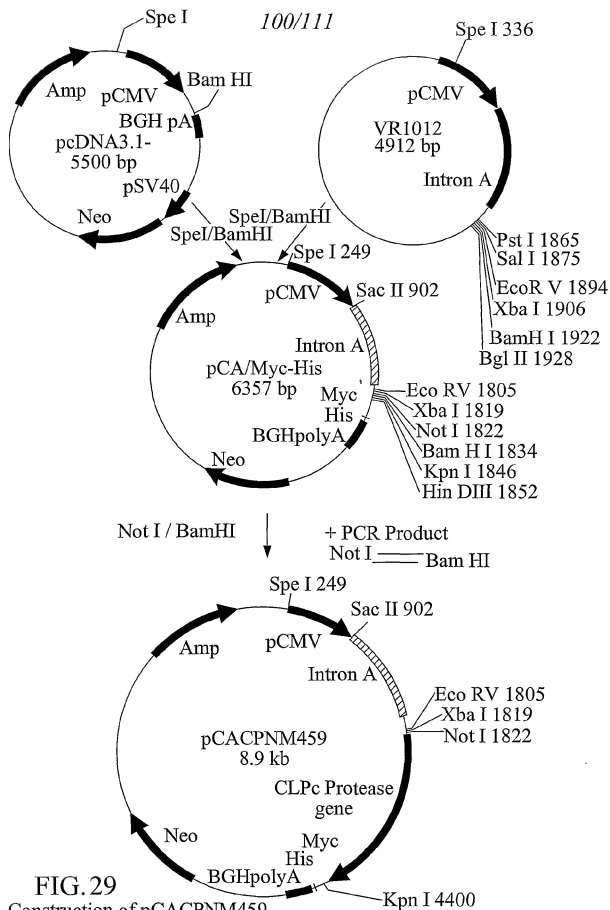
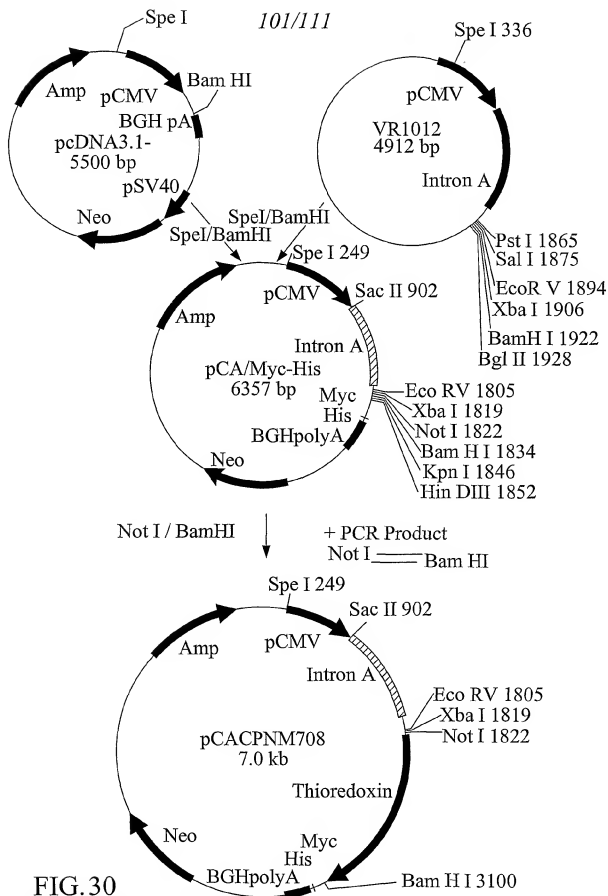


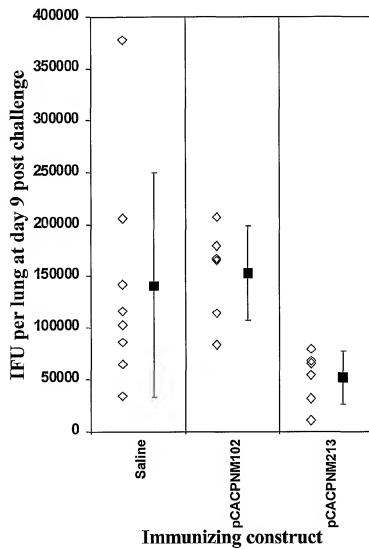
FIG. 29  
Construction of pCACPNM459



**FIG. 30**  
Construction of pCACPNM708

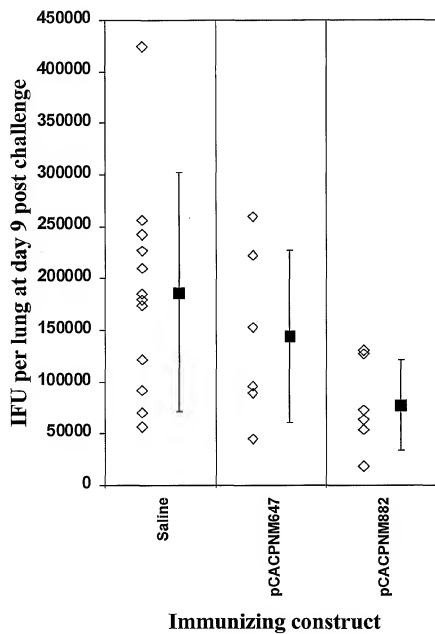
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Figure 31: Protective efficacy of DNA immunization with pCACPMMN213.



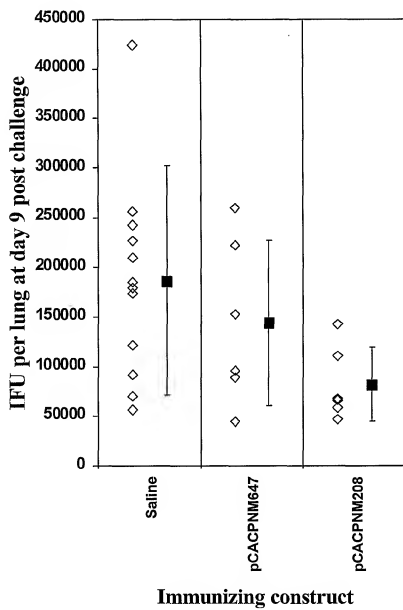
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Figure 32: Protective efficacy of DNA immunisation with pCACPNI882.



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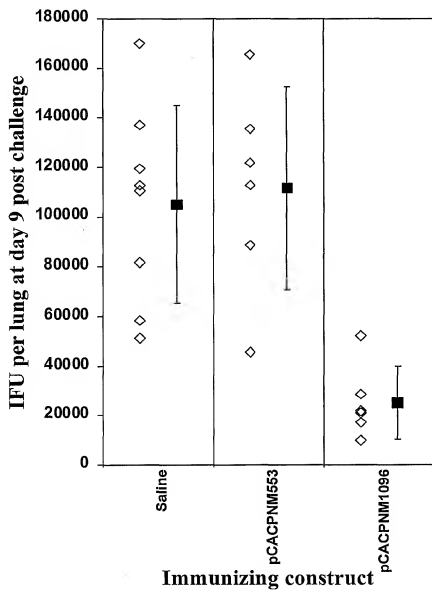
Figure 33: Protective efficacy of DNA Immunisation with pCACPMM208.





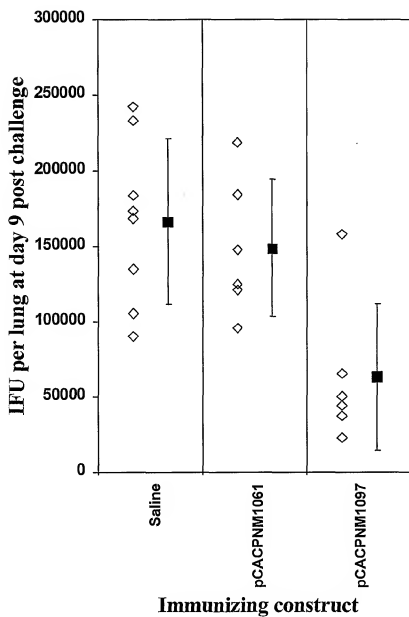
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Figure 34: Protective efficacy of DNA Immunisation with pCACPNI096.



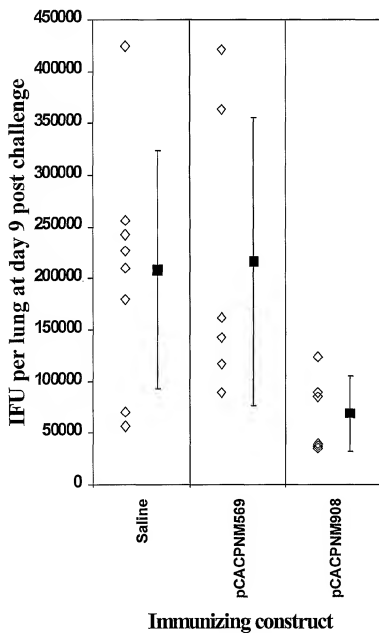
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Figure 35: Protective efficacy of DNA Immunisation with pCACPMM1097.



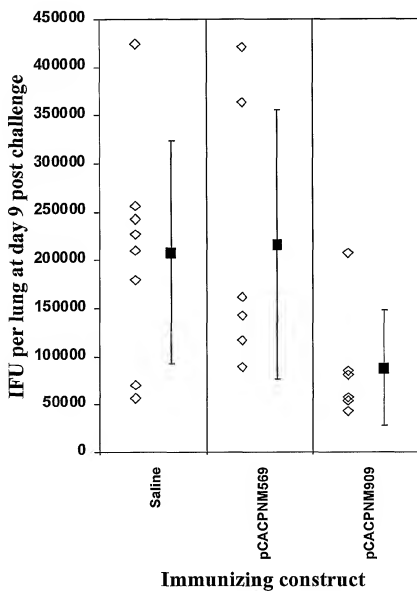
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Figure 36: Protective efficacy of DNA Immunisation with pCACPNM908.



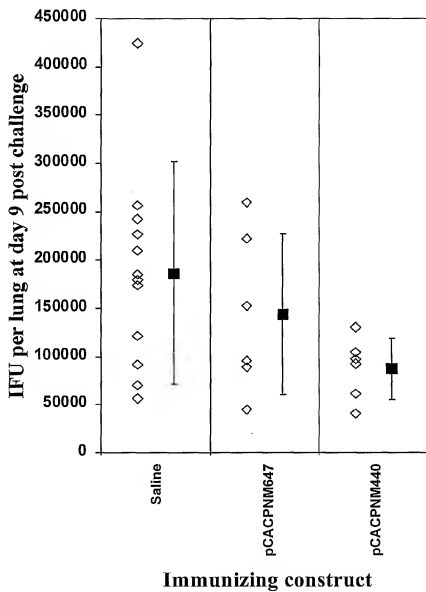
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Figure 37: Protective efficacy of DNA Immunisation with pCACPNM909.



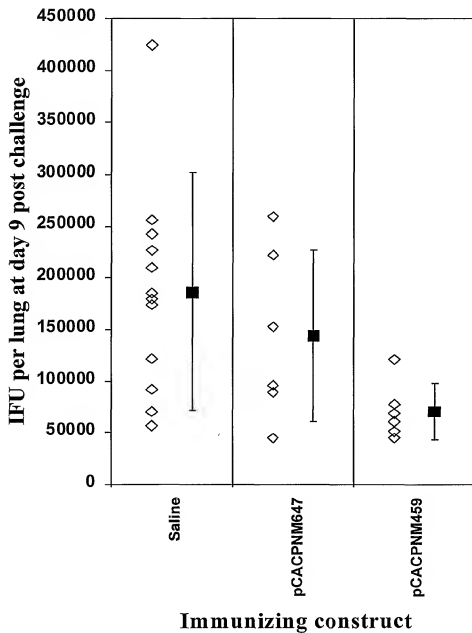
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Figure 38: Protective efficacy of DNA Immunisation with pCACPNM440.



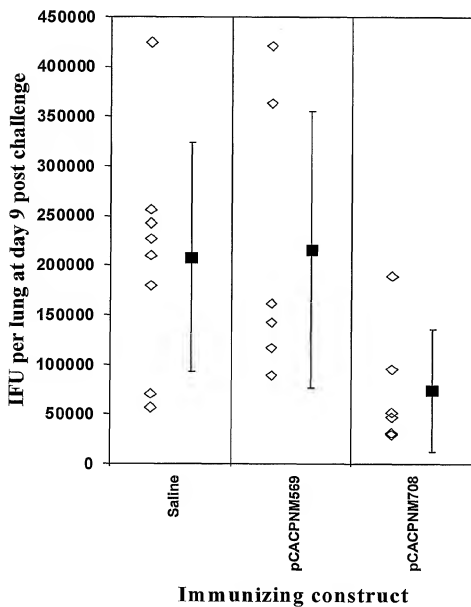
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Figure 39: Protective efficacy of DNA Immunisation with pCACPNI459.



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Figure 40: Protective efficacy of DNA Immunisation with pCACPNI708.



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 Ser Tyr Thr Leu Ser Lys Asp His Lys Val Tyr Thr Phe Lys Leu Arg  
 90 95 100



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Lys Gln Glu Ile Leu Glu Arg Gly Ala Gln Leu Gly Pro Asp Val Ile
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gaa act cta aca ttg cct gag gaa caa gcc gag att ttt tat aaa atg 691
Glu Thr Leu Thr Leu Pro Glu Glu Gln Ala Glu Ile Phe Tyr Lys Met
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	Thr Ser Leu Leu Arg Asp Gly Ile Trp Glu Ala Val Lys Arg Gln Glu	
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	His Glu Ile Val His Asn Arg His Val Val Asn Ala Leu Arg Ala Lys	
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	Gly Ala Ile Phe Val Glu Glu Leu Val Asp Val Pro Glu Gly Glu Arg	
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	Val Ile Tyr Ser Ala His Gly Ile Pro Pro Val Arg Ala Glu Ala	
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	Lys Ala Arg Lys Leu Ile Asp Ile Asp Ala Thr Cys Gly Leu Val Thr	
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	Lys Val His Ser Ala Ala Lys Leu Tyr Ala Ser Lys Gly Tyr Lys Ile	
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	Thr Leu Ser Leu Asp Asp Val Gln Glu Ile Ser Ser Ala Leu Leu Lys	
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	cga tat ccc tct atc att act ctg cct agt tct tcg att tgt tat gca	691
	Arg Tyr Pro Ser Ile Ile Thr Leu Pro Ser Ser Ser Ile Cys Tyr Ala	
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	acc acg aac cgt caa aaa gca ttg cgt tct gtt tta tct cgc gtg aat	739
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	cgc gaa gtg gct ttg aga agg gga gtt ccc gct gat ttg atc aac aat	835
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 Lys Gly Tyr Lys Ile Ile Leu Ile Gly His Lys Lys His Val Glu Val  
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    Gly Ile Ile Tyr Ile Asp Glu Ile Asp Lys Ile Gly Arg Thr Thr Ala
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	Ala Lys Arg Leu Gly Lys Thr Thr Ile Gly Phe Ser Asp Asp Gln Ala	
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 Asp Phe Phe Ala Glu Trp Cys Gly Pro Cys Arg Met Leu Thr Pro Ile  
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tta gaa aat ctt gct gcg gaa ctt cct cat gtc act att gga aaa atc 259  
 Leu Glu Asn Leu Ala Ala Glu Leu Pro His Val Thr Ile Gly Lys Ile  
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10 att cct acg ctt att ctt ttt aag gat ggg aac gag gtg gct cgg gtc 355  
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